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THE INFLUENCE OF NUTRITIONAL DETERMINANTS ON AGEING

DOCTORAL THESIS

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Influencia de determinantes
nutricionales en el envejecimiento

Helena Sandoval Insausti

To my parents and brother

Dr. Pilar Guallar Castillón and Dr. Fernando Rodríguez Artalejo, inform that the thesis entitled *"The influence of nutritional determinants on ageing"* is an original work carried out by Helena Sandoval Insausti under our guidance and supervision. This is an original work and has not been submitted to any university for the awarding of any diploma/degree. We verify that we have read the thesis, that it is well written and it demonstrates a thorough understanding of the scientific methodology.

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LIST OF ABBREVIATIONS

ENRICA: Study on Nutrition and Cardiovascular risk factors in Spain

ORs: odds ratios

CI: confidence interval

MUFAs: monounsaturated fatty acids

SFAs: saturated fatty acids

ALA: α-linolenic acid

LA: linoleic acid

ENRICA: estudio de nutrición y riesgo cardiovascular en España

IC: intervalo de confianza

MIPAA: The Madrid International Plan of Action on Ageing IADLs:

instrumental activities of daily living

RDA: recommended dietary allowance

WHI-OS: Women's Health Initiative Observational Study

MrOS: Osteoporotic Fractures in Men

EPIC: European Prospective Investigation into Cancer and Nutrition

BMI: body mass index

WC: waist circumference

AO: abdominal obesity

SD: standard deviation

Health ABC: Health, Aging and Body Composition

HD: dietary history

MET: metabolic equivalent

MEDAS: Mediterranean Diet Adherence Screener

SUMMARY

The ageing of the population has become a major significant concern and a global public health challenge. Most of the disease burden in the elderly is related to chronic diseases that could be prevented, delayed, or even reversed by focusing on healthy-related behaviours and lifestyle. Even in the very elderly population, an improvement in physical activity, the maintenance of adequate nutrition, and the removal of unhealthy behaviours can have major benefits on well-being. An important example of a syndrome that could be reverted and is associated with an increased risk of hospitalization, disability, morbidity, and mortality, is frailty. Likewise, falling is the most frequent cause of death related to unintentional injury and the six leading cause of death in people aged 65 years or older. However, nutritional determinants associated with frailty and falls have been poorly identified. Therefore, the main objective of this thesis is to address the influence of some of these nutritional determinants on ageing.

Data were taken from the Seniors-ENRICA (Study on Nutrition and Cardiovascular risk factors in Spain) cohort, which was established in 2008-2010 with 2,614 noninstitutionalized aged 60 years and older in Spain. At baseline, a computer-assisted phone interview was performed to collect data on socio-demographic factors, health behaviours and morbidity. Then, two home visits were conducted to obtain a dietary history, perform a physical exam, and collect biological samples; the average time between the phone interview and the second home visit was 2 weeks. For cognitively impaired subjects, data were collected with the help of a proxy. Participants were followed-up until 2012, when a second wave of data collection was performed, including related frailty and falls information. This thesis first evaluates the association of protein and other macronutrient intake with incident frailty in noninstitutionalized older men and women from Spain, using logistic regression models. Then, it assesses the prospective association of protein intake with the risk of falls in the same cohort, and stratifies the analysis according to unintentional weight loss. Logistic models are used to assess the risk of falls. Finally, it investigates the relationship between ultra-processed food intake and incident frailty in the same cohort, using logistic regression models with adjustment for the main confounders.

During a mean follow-up of 3.5 years, 132 participants with incident frailty were identified. The odds ratios (ORs) and the 95% confidence interval (95% CI) of frailty across increasing quartiles of total protein intake were 1.00, 0.55 (0.32-0.93), 0.45 (0.26-0.78), and 0.41 (0.23-0.72); *p* for trend = 0.001. The corresponding figures for animal protein intake were 1.00, 0.68 (0.40-1.17), 0.56 (0.32-0.97), and 0.48 (0.26-0.87); *p* for trend = 0.011. When MUFAs were considered, the results were 1.00, 0.66 (0.37-1.20), 0.54 (0.28-1.02), and 0.50 (0.26-0.96); *p* for trend = 0.38. No association was found between intake of vegetable protein, saturated fats (SFAs), long-chain ω-3 fatty acids, α-linolenic acid (ALA), linoleic acid (LA), simple sugars, or polysaccharides and the risk of frailty. During the same period, a total of 522 participants (21.2%) experienced at least one fall. The ORs (95% CI) of falls for the three increasing tertiles of total protein intake were 1.00, 0.86 (0.66-1.11), and 0.93 (0.70-1.24); *p* for trend = 0.14. However, a statistically significant interaction with unintentional weight loss was observed for the association between protein intake and the risk of falls; *p* for interaction = 0.004. Among 163 participants (6.6%) who experienced an unintentional weight loss of 4.5 kg or more in the preceding year, the ORs (95% CI) of falls for the three increasing tertiles of total protein intake were 1.00, 0.68 (0.21-2.23), and 0.23 (0.05-1.08); *p* for trend = 0.01. Regarding ultra-processed food consumption, expressed as percentage of total energy intake, the ORs (95% CI) for frailty across increasing quartiles were 1.00, 1.52 (0.78-2.96), 2.98 (1.62-5.50), and 3.67 (2.00-6.73); *p* for trend = <0.001. Results were similar when food consumption was expressed as gram per day/kg of body weight (g/kg). A greater intake of ultra-processed foods from non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks), yogurts and fermented milks (natural and non-sugary yogurts are not included in this category), as well as cakes and pastries was also significantly related to incident frailty.

These results provide novel evidence about the influence of the intake of total protein, animal protein, and MUFAs on incident frailty and conclude that promoting the intake of these nutrients might reduce frailty. Additionally, regarding the association of protein intake and falls, high total protein intake shows to confer substantial benefits to participants who experienced an unintentional weight loss of 4.5 kg or more in the preceding year. Finally, the findings add evidence

for the restriction of ultra-processed foods, and the promotion of fresh or minimally processed foods in the older adult population.

RESUMEN

El envejecimiento de la población se ha convertido en una preocupación importante y un desafío mundial de salud pública. La mayor parte de la carga de enfermedad en las personas mayores está relacionada con enfermedades crónicas que podrían prevenirse, retrasarse o incluso revertirse si se enfocan en conductas y estilos de vida relacionados con la salud. Incluso en la población muy anciana, mejorar la actividad física, mantener una nutrición adecuada y eliminar los comportamientos poco saludables tiene grandes beneficios para el bienestar. Un ejemplo importante de un síndrome que podría revertirse y se asocia a un mayor riesgo de hospitalización, discapacidad, morbilidad y mortalidad, es la fragilidad. Asimismo, la caída es la causa más frecuente de muerte relacionada con lesiones no intencionales y la principal causa de muerte en personas con 65 años o más. Sin embargo, los determinantes dietéticos asociados con la fragilidad y las caídas han sido poco identificados. Por lo tanto, el objetivo principal de esta tesis es abordar la influencia de algunos de estos determinantes dietéticos en el envejecimiento.

Los datos se tomaron de la cohorte Seniors-ENRICA (estudio de nutrición y riesgo cardiovascular en España) que se estableció en 2008-2010 con 2614 individuos no institucionalizados de 60 o más años en España. Al inicio, se realizó una entrevista telefónica asistida por ordenador para recopilar datos sobre factores sociodemográficos, comportamientos de salud y morbilidad. A continuación, se llevaron a cabo dos visitas domiciliarias para obtener un historial de dieta, realizar un examen físico y recolectar muestras biológicas; el tiempo promedio entre la entrevista telefónica y la segunda visita domiciliaria fue de 2 semanas. Para los sujetos con deterioro cognitivo, los datos se recopilaron con la ayuda de un proxy. Los participantes fueron seguidos hasta 2012, cuando se realizó una segunda ola de recogida de datos, incluyendo información relacionada con fragilidad y caídas. Esta tesis primero evalúa la asociación entre la ingesta de proteínas así como otros macronutrientes y fragilidad incidente en hombres y mujeres mayores no institucionalizados en España, utilizando modelos de regresión logística. A continuación, evalúa la asociación prospectiva entre la ingesta de proteínas y el riesgo de caídas en la misma cohorte, y estratifica el análisis de acuerdo con la pérdida de peso involuntaria de los participantes. Se utilizan modelos logísticos para valorar el riesgo de caídas. Finalmente, la tesis investiga la relación entre la ingesta de alimentos ultra-procesados y fragilidad

incidente en la misma cohorte, utilizando modelos de regresión logística con ajuste por los principales factores de confusión.

Tras un seguimiento medio de 3,5 años, se identificaron 132 personas con fragilidad incidente. Las ORs y el intervalo de confianza (IC 95%) de fragilidad en los cuartiles crecientes de consumo de proteínas totales fueron 1,00, 0,55 (0,32-0,93), 0,45 (0,26-0,78) y 0,41 (0,23-0,72); *p* de tendencia = 0,001. Las cifras correspondientes a la ingesta de proteínas animales fueron 1,00, 0,68 (0,40-1,17), 0,56 (0,32-0,97) y 0,48 (0,26-0,87); *p* de tendencia = 0,011. Cuando se consideró el consumo de ácidos grasos monoinsaturados, los resultados fueron 1,00, 0,66 (0,37-1,20), 0,54 (0,28-1,02) y 0,50 (0,26-0,96); *p* de tendencia = 0,038. No se encontró asociación entre la ingesta de proteínas vegetales, grasas saturadas, ácidos grasos ω-3 de cadena larga, ácido α-linolénico, ácido linoleico, azúcares simples o polisacáridos y el riesgo de fragilidad. Durante este mismo periodo, un total de 522 participantes (21,2%) experimentaron al menos una caída. Las ORs (IC 95%) de caídas en los tres terciles crecientes de ingesta total de proteínas fueron 1,00, 0,86 (0,66-1,11) y 0,93 (0,70-1,24); *p* de tendencia = 0,14. Sin embargo, se observó una interacción estadísticamente significativa con la pérdida de peso involuntaria para la asociación entre la ingesta de proteínas y el riesgo de caídas; *p* de interacción = 0,004. Entre los 163 participantes (6,6%) que experimentaron una pérdida de peso involuntaria de 4,5 Kg o más en el año precedente, las ORs (IC 95%) de caídas en los tres terciles crecientes de ingesta de proteínas totales fueron 1,00, 0,68 (0,21-2,23) y 0,23 (0,05-1,08); *p* de tendencia = 0,01. En lo referente al consumo de ultra-procesados, expresado como porcentaje de la ingesta total de energía, las ORs (IC 95%) en los cuartiles crecientes de fragilidad fueron 1,00, 1,52 (0,78-2,96), 2,98 (1,62-5,50), y 3,67 (2,00-6,73); *p* de tendencia = <0,001. Los resultados fueron similares cuando el consumo de alimentos se expresó en gramos por día/kg de peso corporal (g/kg). Un mayor consumo de alimentos ultra-procesados procedente de las bebidas no alcohólicas (café y cacao instantáneo, zumos envasados y otras bebidas no alcohólicas excluyendo los refrescos), los yogures y las leches fermentadas (los yogures naturales y los no azucarados no están incluidos en esta categoría), así como las tartas y la pastelería, también se relacionó significativamente con fragilidad incidente.

Estos resultados proporcionan nueva evidencia sobre la influencia de la ingesta de proteínas totales, proteínas animales y ácidos grasos monoinsaturados en la fragilidad incidente y concluyen que promover la ingesta de estos nutrientes podría reducir la fragilidad. Además, con respecto a la asociación de la ingesta de proteínas y las caídas, la alta ingesta de proteínas totales muestra beneficios sustanciales en los participantes que experimentaron una pérdida de peso no intencional de 4,5 kg o más en el año previo. Finalmente, los hallazgos agregan evidencia sobre la restricción de los alimentos ultra-procesados y la promoción de alimentos frescos o mínimamente procesados en la población de adultos mayores.

1. GENERAL INTRODUCTION

1.1. Ageing

The ageing of the population has become a major significant concern and a global public health challenge. In the next few years, adults aged 65 years and older will exceed the number of children aged 5 years or younger. The age pyramid has changed into an older population structure in European countries due to the decrease in birth rates and the continued increase in life expectancy (1). Since the 1970s, the main factor contributing to the increase in life expectancy in high-income countries has been the decline in mortality among the elderly, specifically deaths from non-communicable diseases, such us diseases associated with cardiovascular disease.

The population of the European Union was estimated at 512.6 million on 1 January 2018, compared with 511.5 million on 1 January 2017 (2). In 2017, the distribution of the population was the following: 15.6% were young people (0 to 14 years old), 64.9% were of working age (15 to 64 years old), 29.4% were older adults (aged 65 years or older). Between 2002 and 2017, the median age increased by 4.2 years, from 38.6 years to 42.8 years. The comparison of age pyramids for 2017 and 2080 shows that the population is expected to continue to age. By 2080, the pyramid will have the shape of a block, being considerably smaller around the age 45–54 years old (1). Between 2017 and 2080, the percentage of those aged 80 years or older is projected to rise from 5.5 % to 12.7 % (1).

A steady rise in the older population demands a major public health response and also requires health systems to find effective strategies to respond to their needs (3). In 2002, the United Nations General Assembly endorsed The Madrid International Plan of Action on Ageing (MIPAA) (4). They identified priorities for action including promotion of health and well-being in the elderly, universal and equal access to health-care services, training of care providers and health professionals, and appropriate services for older persons with disability.

Most of the disease burden in the elderly are related to chronic diseases that could be prevented, delayed, or even reversed by focusing on healthy-related behaviours and lifestyle throughout lifespan (5). Unhealthy behaviours have a large variation among countries suggesting that many opportunities exist for intervention.

Even in the very elderly population, to improve physical activity, to maintain adequate nutrition, and to remove unhealthy behaviours can have major benefits on their well-being (6-8). Two of the most effective strategies in older adults are related to improving nutrition (5, 9), as well as stopping smoking (10).

1.2. Frailty

Frailty is a multidimensional impairment characterized by decreased functional reserve (11, 12), and increased difficulty to integrate adequate responses even to minor stressors, that could result in adverse health outcomes such as disability, hospitalization, care home admission, and mortality (13, 14). Frail individuals are vulnerable to changing their health status from independent to dependent due to minor events in a relative short period of time (15).

Rockwood described frailty as an accumulation of deficits in multiple inter-related ageing systems (described in the 70-Item CSHA Frailty Index developed by Rockwood et al) (16). These complex ageing systems are influenced by genetic and environmental factors in combination with epigenetic mechanisms, which regulate the differential expression of genes in cells and have an important influence on ageing (17). On the other hand, Fried et al defined frailty as a medical syndrome (assessed as the Fried frailty phenotype), with sarcopenia, defined as skeletal muscle loss, as its pathological basis (12). This is the most frequently used definition and focuses on the evaluation of five domains (nutritional status, energy, physical activity, mobility, and strength), establishing five criteria (one per each domain: low physical activity, slowness, unintentional weight loss, muscle weakness, and exhaustion) (12). In addition to assessing physical functioning, other researchers include in the frailty definition other domains such as cognition, mood, and other aspects related to mental health (18). Likewise, Rodríguez-Mañas et al pointed out that frailty definitions should be validated in different cultural, economic, and ethnic settings (19-21).

A recent systematic review assessed the prevalence of frailty in community-dwelling adults aged 65 and older (22). They found a frailty prevalence of 9.9% and a pre-frailty prevalence of 44.2%. Frailty was more prevalent in women (9.6%) than in men (5.2%), and increased continuously with age: the prevalence of frailty is 4%

in ages 65-69 years old; 7% in ages 70-74 years old; 9% in ages 75-79 years old; 16% in ages 80-84 years old; and 26% in ages 85 years or older (15). It is estimated that from a quarter to half of people over 85 years are frail (12, 23). Frailty is a dynamic process, potentially reversible, but a transition to a level of greater frailty is the most common scenario (24). Reducing the prevalence or severity of frailty will have profound health and social implications. Multidisciplinar approaches are being investigated: exercise interventions, nutritional interventions, and pharmacological agents, that benefit the elderly and their families, as well as reducing the burden associated with health and social care (15).

1.3. Falls

Falls are a major public health problem among the elderly (25-28). In fact, falling is the leading cause of death related to unintentional injury (27, 29), and the six most frequent cause of death in 65 years or older (25, 30). More than one in three community-dwelling adults aged 65 years or older have experienced at least one fall in the previous year (25, 27, 31), and in half of the cases the falls are recurrent (25, 29). This risk of falling exceeds 35% per year among those aged 75 years and older (29). Moreover, it is sometimes difficult to reconstruct the events underlying the fall, even in the absence of a cognitive impairment, so the incidence of falls could be underestimated (32).

Approximately 1 out of 10 falls in the elderly results in serious injuries (hip fracture, head injury, subdural hematoma…) (29, 33). Independently of the older adult's health status, falls are linked to loss of functional capacity and a decline in the ability to carry out instrumental activities of daily living (IADLs) such as dressing, bathing, shopping, or housekeeping (34, 35). Falls are also associated with an increased risk of institutionalization, health care services use, and mortality (26, 31, 32).

Although some falls have a single cause, the pathogenesis of most of them is multifactorial and complex (31, 32). The identification of risk factors is essential in order to develop effective preventive measures (36). Medical and environmental risk factors have been identified (37-39). The following conditions have been shown to increase the risk of falling: arthritis, depression, cognitive dysfunction, loss of

vision, balance impairment, gait abnormalities, decreased muscle strength, poor general health, poor dental health, and daily polypharmacy (31, 40). However, potential dietary determinants have been poorly identified (41). More studies are needed to identify these determinants on the way to develop strategies focus on reducing the frequency of falls and their consequences (25, 41-43).

1.4. Nutrition

Ageing, as a result of the accumulation of molecular and cellular damage, is accompanied by physiological changes as well as psychosocial and environmental challenges, that can be associated with malnutrition (3).

Sensory impairments, poor oral health (44, 45), gastric acid secretion deficiency, loss of vision and hearing, arthritis, isolation, depression, and inadequate finances, are some of the factors which impact negatively on nutrition in the elderly (3).

Malnutrition of both macro- and micronutrients is associated with a decreased muscle mass (46, 47) and with an increased risk of frailty in older adults (48). Likewise, overall diet quality has been linked to a lower risk of frailty. Compared with participants in the lowest quartile, those in the highest quartile of the Mediterranean diet score showed a significantly reduced risk of being frail (25). Malnutrition has also been related to an increased risk of cognitive impairment and becoming care-dependent (3). Although energy needs diminish with age due to fewer caloric requirements, the risk of malnutrition is exacerbated because the need for most nutrients remains relatively unaltered (3), primarily in older people with increased health care needs (48).

However, malnutrition is frequently underdiagnosed in older adults. As a consequence, the global prevalence of malnutrition is not well established, though evidence suggests it is significant (3). In the United Kingdom, the risk of protein–energy malnutrition was established between 11% and 19%, and it was accompanied by deficiencies of vitamins C and D, and low levels of carotenoids (49).

The management of malnutrition in the elderly should be multidimensional.

A correct nutritional assessment requires performing anthropometric measurements, clinical samples, and a dietary history (3). A randomized controlled trial showed that different interventions are effective in reversing malnutrition and delaying dependency such as improving fluid, protein, and energy intake, doing exercise to build strength, and increasing IADLs (50). Therefore, energy and protein intake is an important factor where interventions should focus on. Further studies are needed to develop new multifaceted interventions aimed at improving nutrition in the elderly.

1.5. Protein intake

Inadequate protein intake is an important factor that causes skeletal muscle depletion by reducing the synthesis rather than increasing the degradation of muscle protein (51, 52). Moreover, protein intake has been associated with preservation of muscle mass (53, 54). Specifically, individuals in the highest quintile of daily protein intake (1.20 g/kg/d) lost 40% less total body and appendicular lean mass than those in the lowest quintile (0.7 g/kg/d) (53). In addition, muscle strength plays a key role in the risk of falls, which may result in fractures and other important injuries (55). Protein intake has also been associated with a lower risk of incident frailty (56). However, no previous epidemiological study has focused on the association between other macronutrients and the risk of frailty.

There is some concern that consuming dietary protein in excess of the current recommended dietary allowance (RDA) may cause renal damage (57). However, there is no evidence for a detrimental effect of high protein intake much above the RDA in healthy adults without alteration of the renal function (57, 58). Taking into account that the threshold for anabolic intake of protein is greater in the elderly due to an attenuated stimulation of protein synthesis by essentials amino acids (51, 59), the new dietary recommendations support a moderate increase of protein intake from 0.8 to 1.0–1.2 g/kg/day (60-62).

1.6. Ultra-processed food intake

Ultra-processed foods are defined as food or drink products originating from whole foods extraction or refined, with little or no whole food content. These foods include extracted or refined oils, hydrogenated oils and fats, protein isolates, flours and starches, variants of sugar, and remnants of animal foods (63-65). They are energy dense and have high content of total fat, saturated fat, trans fat, simple sugar, and salt, but low content of fiber and vitamin density (66-69).

The NOVA classification established the ultra-processed foods concept. It classified foods regarding the extent of their industrial food processing (66, 70). NOVA classification includes four groups: (i) unprocessed or minimally processed foods, defined as vegetable or animal-origin foods consumed shortly after harvesting, gathering, slaughtering or husbanding; or foods which involve food parts subtraction without the introduction or addition of any substances. Fruit and vegetables, grains, nuts and seeds, fresh and pasteurized milk, and natural yogurt with no added sugar or artificial sweeteners, are some examples of foods belonging to this group; (ii) processed culinary ingredients, defined as food products extracted and refined from constituents of foods or obtained from nature, but not consumed by themselves. For example: salt, sugar, honey, vegetable oils, butter, lard, and vinegar; (iii) processed foods are unprocessed or minimally processed foods altered with the addition of salt or sugar (or other substance of culinary use such as oil or vinegar), in order to preserve them or to enhance their palatability. Canned or bottled vegetables and legumes, fruit in syrup, canned fish, unpackaged cheeses, freshly made bread, and salted nuts and seeds, are in this group; and (iv) ultra-processed foods. Food or drink products formulated mostly or entirely from substances derived from foods and additives, with little if any unaltered food. Some examples of ultra-processed foods are burgers, frozen pizza and pasta dishes, nuggets and fish fingers, crisps, mass-manufactured breads and cakes, biscuits, jams and confectionery, margarines, cereal bars, carbonated and other sugary drinks, sugary or sweetened milks, and various snack products (63-65, 71, 72).

Most ultra-processed foods are made and advertised by large corporations and are durable, affordable, and ready to eat, drink, or heat, that is an advantage over fresh and perishable foods (64, 68). As a consequence, ultra-processed food intake is substantially growing worldwide (63). There is evidence that ultra-processed food

consumption promotes overweight/obesity (73, 74), increases the risk of hypertension (72), high total cholesterol and low-density lipoprotein cholesterol levels in children (75), metabolic syndrome in adolescents (76), cancer (77), and other chronic diseases (78). However, no previous epidemiological study has focused on the association between ultra-processed foods and frailty.

2. OBJECTIVES

OBJECTIVE 1: To assess the association between macronutrient intake and the risk of frailty in adults aged 60 years or older. Specifically, to assess the role of:

1.1: Total protein, animal protein, and vegetable protein intake.

1.2: Monounsaturated fatty acids, saturated fats, long-chain ω-3 fatty acids, α-linolenic acid, and linoleic acid.

1.3: Simple sugars and polysaccharides.

1.4: The recent recommendation of protein intake averaging 1.20 g/kg/d.

OBJECTIVE 2: To examine the association between protein intake and the risk of falls in adults aged 60 years or older. Specifically, to examine the role of:

2.1: Total protein intake.

2.2: Animal protein and vegetable protein intake separately.

2.3: Recent unintentional weight loss on the study association.

OBJECTIVE 3: To evaluate the association between consumption of ultra-processed foods and the risk of frailty in adults aged 60 years or older. Specifically to evaluate the role of:

3.1: Total ultra-processed food consumption.

3.2: The consumption of the main groups of ultra-processed foods.

3. Macronutrients Intake and Incident Frailty in Older Adults: A Prospective Cohort Study

3.1 INTRODUCTION

Frailty is a medical syndrome characterized by sarcopenia, decreased functional reserve, and augmented vulnerability to even minor stressors (12). As a result, frail individuals are at increased risk of disability, institutionalization, and death (15). Due to the progressive ageing of the population in industrialized countries, prevention strategies should focus on delaying frailty, reversing the frailty process, and reducing the adverse health outcomes of the frailty syndrome (13).

Poor nutrition plays a key role in the frailty process (48). However, research on the relationship between macronutrient intake and incident frailty is scarce. To our knowledge, only two cohort studies have assessed this association. The first one is the Women's Health Initiative Observational Study (WHI-OS) (56), conducted with 24,417 women aged 65 or older. In this study, a 20% higher protein intake was associated with a 32% lower risk of frailty in the subsequent three years. This association was independent of the source (animal or vegetable) and quality (essential amino acid intake) of protein. However, no information on other macronutrients was reported. The second study is the Osteoporotic Fractures in Men (MrOS) study (79), where no association was found between carbohydrate, fat, or protein intake and risk of frailty among 2,423 men aged 65 or older followed during 4.6 years. However, the statistical power of this analysis was limited because only 25 frail men were identified.

Therefore, this study examined the association of protein and other macronutrient intake with the risk of frailty in noninstitutionalized older men and women from Spain.

3.2. METHODS

3.2.1. Study design and Population

Data were taken from the Seniors-ENRICA cohort, which was established in 2008-2010 with 2,614 community-dwelling individuals aged 60 years or older in Spain (80, 81). At baseline, a computer-assisted phone interview was performed to

collect data on socio-demographic factors, health behaviours, and morbidity. Then, two home visits were conducted to obtain a diet history, perform a physical exam and collect biological samples; the average time between the phone interview and the second home visit was 2 weeks. For cognitively impaired participants, data were collected with the help of a proxy. Participants were followed-up until 2012, when a second wave of data collection was performed. Ninety-five participants (3.6%) died during follow-up; from the remaining 2,519 participants, 2,085 had complete information on frailty (80, 81). Of them, we excluded 174 for being frail or having missing values for frailty at baseline, 8 with unreliable information on diet, and 81 who lacked data on diet or potential confounders. Thus, the analyses were performed with 1,822 subjects. Compared with individuals included in the analyses, those not included were less often males, were slightly older, and had more frequent chronic conditions (Supplementary Table 1).

Participants gave written informed consent. The study was approved by The Clinical Research Ethics Committee of "La Paz" University Hospital in Madrid (Spain).

3.2.2. Study Variables

3.2.2.1. Diet

At baseline, habitual food consumption was collected through a validated computerized face-to-face diet history (82), which was developed from that used in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Spain cohort study. Collected information included all foods consumed at least once every 15 days during the preceding year. The diet history registers 860 foods and 30 different cooking methods and uses more than 120 photographs to help in choosing the portion sizes. The intake of animal protein, vegetal protein, MUFAs, SFAs, long-chain ω-3 fatty acids, ALA, LA, simple sugars, and polysaccharides was estimated with standard food composition tables.

3.2.2.2. Frailty

Frailty was assessed according to the definition developed by Fried and

colleagues (12). Specifically, frailty was defined as having at least three of the following five criteria: (i) low physical activity, defined as walking ≤2.5 h/week in men and ≤2 h/week in women; (ii) slowness, defined as the lowest quintile in our study cohort for the 3-m walking speed test, adjusted for sex and height; in men, the cut-off points were 0.47 and 0.45 m/second in those with height ≤173 cm and >173, respectively; corresponding values in women were 0.37 and 0.40 m/second; (iii) unintentional weight loss, evaluated as involuntary loss of 4.5 kg (10 lb) or more in the preceding year; (iv) muscle weakness, defined as the lowest quintile of grip strength within groups defined by sex and body mass index (BMI) in our cohort. Strength was measured with a Jamar dynamometer, and we selected the highest value in two consecutive measurements; in men, the cut-off points were <29, <30 and <32 kg, respectively, in those with BMI ≤24, 24.1-28, and >28 kg/m^2; the corresponding values in women were <17, <17.3, <18, and <21 kg, respectively, in those with BMI ≤23, 23.1-26, 26.1-29, and >29 kg/m^2; and (v) exhaustion, defined as responding "at least 3 to 4 days a week" to any of the following two questions taken from the Center for Epidemiologic Studies-Depression scale (83): "I felt that anything I did was a big effort" or "I felt that I could not keep on doing things".

3.2.2.3. Other Variables

At baseline, data on other socio-demographic factors, lifestyle, and morbidity were collected. Participants reported their sex, age, level of education, marital status, tobacco consumption, leisure-time physical activity (84), and physical activity in the household (84). Weight, height, and waist circumference (WC) were measured under standardized conditions. BMI was calculated as weight in kilograms divided by square height in meters. Abdominal obesity (AO) was defined as WC greater than 102 cm in men and greater than 88 cm in women. Study participants also reported whether they suffered from any of the following physician-diagnosed prevalent diseases: chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, diabetes, and depression requiring treatment. Participants also reported the number of medications used, which was checked against the drug packages. Finally, their ex-drinker status was self-reported.

3.2.3. Statistical Analysis

The associations between macronutrient intake and frailty were summarized with ORs (95% CI), obtained from logistic regression. The dose-response relationship was tested with a *p* for linear trend, obtained by modeling quartiles of nutrient intake as a continuous variable. Analyses were energy adjusted with the nutrient residual model (85). Dietary variables were log transformed (86), and the residuals were obtained by regressing nutrient intake on total energy. The residuals of all sources of energy were then included in the model except for carbohydrates. Total energy intake was also included as a covariate. In this residual model, the coefficient for a certain nutrient is interpreted as the effect of substituting the amount of energy proceeding from such nutrient for the same amount of energy from carbohydrates, while holding constant total energy intake and the intake of other macronutrients in the model (85). Conversely, to assess the association with carbohydrates, protein intake was excluded from the model. Macronutrients were modeled as sex-specific quartiles.

Three models were built with progressive adjustment for the main confounders. We selected the potential confounders a priori, because they were associated with either diet or frailty in the literature. The first model was adjusted for sex, age (60-69, 70-79, 80 years and older), total energy intake (kcal/d), the studied macronutrients, and ethanol intake (sex-specific quartiles). Model 2 was additionally adjusted for education (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (never, former, current smoker), BMI (<25, 25-29.9, ≥30 kg/m^2), AO, and fiber intake (sex-specific quartiles). Finally, Model 3 was further adjusted for chronic morbidity: chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, diabetes, depression requiring treatment, and ex-drinker status.

Finally, among participants who were free of all frailty criteria at baseline, we assessed the association between macronutrient intake and each frailty criterion, using the same type of modeling.

Statistical significance was set at two-sided $p < 0.05$. The analyses were conducted with Stata/SE, version 11.1 (StataCorp, College Station, TX).

3.3. RESULTS

The mean age of participants was 68.7 years, standard deviation (SD) = 6.4 years, and 48.7% were men. Over a mean follow-up of 3.5 years, 132 individuals with incident frailty were identified. Compared with non-frail individuals, those with frailty were less often males, were older, had a higher educational level, were more likely to smoke tobacco, and showed a higher frequency of obesity and other chronic conditions (Supplementary Table 2). Also, they had lower intake of energy and of most nutrients (Supplementary Table 3). Supplementary Table 4 shows the baseline characteristics of the study participants according to the studied macronutrients.

In fully adjusted analyses, the ORs (95% CI) of frailty across increasing quartiles of total protein were 1.00, 0.55 (0.32-0.93), 0.45 (0.26-0.78), and 0.41 (0.23-0.72); *p* for trend = 0.001. As in most nutritional analyses, the explained variance was low. Specifically, the proportion of the variance of frailty risk explained by total protein was 1.30%. The ORs (95% CI) of frailty risk across quartiles of animal protein intake were 1.00, 0.68 (0.40-1.17), 0.56 (0.32-0.97), and 0.48 (0.26-0.87); *p* for trend = 0.011. The proportion of the variance of frailty risk explained by animal protein intake was 0.79%. There was an inverse trend between vegetable protein intake and the risk of frailty, although the ORs did not reach statistical significance (Table 1).

When MUFAs were considered, the ORs (95% CI) for frailty according to increasing quartiles of intake were 1.00, 0.66 (0.37-1.20), 0.54 (0.28-1.02), and 0.50 (0.26-0.96); *p* for trend = 0.038 (Table 2). The proportion of the variance of frailty risk explained by MUFA intake was 0.36%.

To assess the robustness of the results, we ran a number of sensitivity analyses. Results for protein and MUFA intake were similar when a loss of body weight of 5% or more was used as frailty criterion, when we removed fiber intake from Model 2 to rule out overadjustment, when protein intake was expressed as g/kg/d, and when we excluded AO from Model 2 to exclude the influence of muscle lipid accumulation on frailty. Finally, we did not find an interaction between macronutrient intake and age (<75 versus ≥75 years), based on likelihood ratio tests

across quartiles of nutrient intake. The corresponding *p* values for interaction were 0.79 for total protein intake, 0.59 for animal protein intake, and 0.30 for MUFA intake.

No statistically significant association was found between incident frailty and intake of SFAs, long-chain ω-3 fatty acids, ALA or LA (Table 2). Neither was it observed for total carbohydrates, simple sugars, or polysaccharides (data not shown). Results were similar in the sensitivity analyses (data not shown).

Regarding the frailty components, the OR (95% CI) of slow walking speed among those in the highest versus the lowest quartile of total protein intake was 0.63 (0.41-0.96); *p* for trend = 0.022. The corresponding figure for animal protein intake was 0.45 (0.30-0.69); *p* for trend = <0.001. Likewise, MUFA intake was inversely associated with unintentional weight loss; the OR (95% CI) was 0.45 (0.24-0.82) for the highest versus the lowest quartile; *p* for trend= 0.012 (Supplementary Table 5). Results were also in the same direction for the rest of the frailty criteria, except for muscle weakness.

Table 1. Odds ratios (95% confidence interval) of incident frailty according to quartiles of protein intake in the Seniors-ENRICA cohort study (*N*=1,822).

	Frailty cases / *n*	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Total protein				
Quartile 1 (lowest)	38/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	37/455	0.60 (0.37-0-98)*	0.62 (0.37-1.03)	0.55 (0.32-0.93)*
Quartile 3	31/456	0.48 (0.29-0.81)†	0.51 (0.30-0.88)*	0.45 (0.26-0.78)†
Quartile 4 (highest)	26/455	0.47 (0.27-0.79)†	0.45 (0.26-0.79)†	0.41 (0.23-0.72)†
p Value for trend		0.002	0.003	0.001
Animal protein				
Quartile 1 (lowest)	35/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	38/455	0.76 (0.46-1.26)	0.76 (0.45-1.27)	0.68 (0.40-1.17)
Quartile 3	29/456	0.64 (0.38-1.08)	0.61 (0.35-1.04)	0.56 (0.32-0.97)*
Quartile 4 (highest)	30/455	0.56 (0.32-0.97)*	0.52 (0.29-0.93)*	0.48 (0.26-0.87)*
p Value for trend		0.030	0.019	0.011
Vegetable protein				
Quartile 1 (lowest)	32/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	36/455	0.69 (0.41-1.18)	0.82 (0.46-1.45)	0.76 (0.42-1.37)
Quartile 3	32/456	0.72 (0.42-1.23)	0.95 (0.52-1.74)	0.87 (0.47-1.63)
Quartile 4 (highest)	32/455	0.45 (0.25-0.82)†	0.70 (0.35-1.43)	0.65 (0.31-1.34)
p Value for trend		0.002	0.003	0.001

Notes: OR = odds ratio; CI = confidence interval.

The *p* values for the OR were obtained using logistic regression:* $p < 0.05$; † $p < 0.01$.

Model 1: Logistic regression model adjusted for sex, age (60-69, 70-79, 80 years or older), energy intake (quartiles), ethanol (quartiles), lipids (quartiles), and for animal protein or vegetable protein as appropriate.

Model 2: As in Model 1 and additionally adjusted for level of education (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (never, former and current smokers), body mass index (<25, 25-29.9, ≥30 kg/m^2), abdominal obesity (yes, no), and dietary fiber (quartiles).

Model 3: As in Model 2 and additionally adjusted for chronic respiratory disease (yes, no), coronary disease (yes, no), stroke (yes, no), osteoarthritis/arthritis (yes, no), cancer (yes, no), diabetes (yes, no), depression (yes, no), number of medications (continuous), and ex-drinker status (yes, no).

Table 2. Odds ratios (95% confidence interval) of incident frailty according to quartiles of fatty acids intake in the Seniors-ENRICA cohort study (*N*=1,822).

	Frailty cases/ *n*	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Monounsaturated fatty acids				
Quartile 1 (lowest)	40/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	29/455	0.77 (0.44-1.33)	0.69 (0.39-1.23)	0.66 (0.37-1.20)
Quartile 3	35/456	0.65 (0.36-1.18)	0.58 (0.31-1.07)	0.54 (0.28-1.02)
Quartile 4 (highest)	28/455	0.59 (0.32-1.10)	0.50 (0.26-0.96)*	0.50 (0.26-0.96)*
p Value for trend		0.09	0.039	0.038
Saturated fatty acids				
Quartile 1 (lowest)	25/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	31/455	1.06 (0.57-0.97)	0.88 (0.47-1.67)	0.90 (0.46-1.74)
Quartile 3	38/456	1.82 (1.00-3.31)	1.49 (0.78-2.85)	1.72 (0.89-3.35)
Quartile 4 (highest)	38/455	1.56 (0.83-2.95)	1.25 (0.62-2.53)	1.28 (0.62-2.63)
p Value for trend		0.08	0.32	0.27
Long-chain ω-3 fatty acids				
Quartile 1 (lowest)	44/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	39/455	0.72 (0.44-1.17)	0.80 (0.48-1.32)	0.72 (0.43-1.21)
Quartile 3	23/456	0.56 (0.32-0.97)*	0.59 (0.33-1.04)	0.62 (0.34.1.12)
Quartile 4 (highest)	26/455	0.68 (0.38-1.22)	0.75 (0.41-1.37)	0.79 (0.43-1.46)
p Value for trend		0.09	0.18	0.30
Alpha-linolenic acid				
Quartile 1 (lowest)	35/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	33/455	0.77 (0.44-1.34)	0.86 (0.48-1.55)	0.87 (0.48-1.58)
Quartile 3	39/456	0.94 (0.53-1.69)	1.04 (0.57-1.90)	1.04 (0.56-1.93)
Quartile 4 (highest)	25/455	0.61 (0.33-1.16)	0.69 (0.36-1.33)	0.71 (0.36-1.39)
p Value for trend		0.23	0.40	0.45
Linoleic acid				
Quartile 1 (lowest)	27/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	37/455	1.48 (0.81-2.70)	1.56 (0.84-2.90)	1.64 (0.86-3.12)
Quartile 3	41/456	2.32 (1.24-4.33)†	2.38 (1.25-4.54)†	2.29 (1.18-4.45)*
Quartile 4 (highest)	27/455	1.58 (0.81-3.09)	1.84 (0.92-3.67)	1.84 (0.90-3.77)
p Value for trend		0.13	0.06	0.08

Notes: OR = odds ratio; CI = confidence interval.

The *p* values for the OR were obtained using logistic regression:* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$. Models adjusted as in Table 1 including the other fatty acids in the table and total protein intake (quartiles).

Supplementary Table 1. Baseline characteristics of the participants included and not included in the analyses (*N*=2,614).

	Included in the analyses (*N*=1,822)	Not included in the analyses (*N*=792)	*p* Value for trend*
Men (%)	48.7	43.7	0.017
Age, years (mean, SD)	68.7 (6.4)	69.6 (6.8)	0.001
Level of education (%)			
Primary or less	21.5	18.4	0.025
Secondary	24.9	22.2	
University	53.6	59.3	
Marital status (%)			
Single	6.8	7.1	0.034
Married	72.6	67.3	
Divorced	3.2	3.6	
Widower	17.3	22.0	
Tobacco consumption (%)			
Never smoker	11.6	10.6	0.49
Former smoker	30.9	29.4	
Current smoker	57.5	60.0	
Body mass index (kg/ m^2) (%)			
<25	19.5	20.1	0.10
25-29.9	49.9	45.5	
≥30	30.6	34.4	
Abdominal obesity (%)	57.0	59.2	0.30
Chronic respiratory disease (%)	7.6	7.7	0.91
Coronary disease (%)	1.4	2.3	0.12
Stroke (%)	0.9	1.9	0.040
Osteoarthritis/arthritis (%)	47.1	53.4	0.003
Cancer (%)	1.9	2.8	0.14
Diabetes (%)	15.1	18.4	0.033
Depression (%)	7.4	10.4	0.012
Number of medications (mean, SD)	2.0 (1.9)	2.2 (1.9)	0.001
Ex-drinker status (%)	9.6	11.9	0.08

Notes: SD = standard deviation.

The T test was used for the comparison of two means. The Chi-square test was used for comparing percentages.

Supplementary Table 2. Baseline characteristics of the study participants according to frailty status (*N*=1,822).

	Total (*N*=1,822)	Non frail (*N*=1,690)	Frail (*N*=132)	*p* Value for trend*
Men (%)	48.7	50.3	28.8	<0.001
Age, years (mean, SD)	68.7 (6.4)	68.3 (6.1)	73.8 (7.4)	<0.001
Level of education (%)				
Primary or less	21.5	22.4	9.9	<0.001
Secondary	24.9	25.9	12.1	
University	53.6	51.7	78.0	
Marital status (%)				
Single	6.8	6.8	7.6	<0.001
Married	72.6	73.8	57.6	
Divorced	3.2	3.3	2.3	
Widower	17.3	16.2	32.6	
Tobacco consumption (%)				
Never smoker	11.6	12.1	5.3	0.026
Former smoker	30.9	31.1	28.0	
Current smoker	57.5	56.8	66.7	
Body mass index (kg/m^2) (%)				
<25	19.5	20.2	10.6	<0.001
25-29.9	49.9	50.8	38.6	
≥30	30.6	29.1	50.8	
Abdominal obesity (%)	57.0	55.2	81.1	<0.001
Chronic respiratory disease (%)	7.6	7.5	9.1	0.49
Coronary disease (%)	1.4	1.2	3.8	0.018
Stroke (%)	0.9	0.8	2.3	0.10
Osteoarthritis/arthritis (%)	47.1	45.4	68.2	<0.001
Cancer (%)	1.9	1.8	3.0	0.31
Diabetes (%)	15.1	14.1	28.0	<0.001
Depression (%)	7.4	6.8	15.9	<0.001
Number of medications (mean, SD)	2.0 (1.9)	1.9 (1.9)	3.0 (2.3)	<0.001
Ex-drinker status (%)	9.6	9.4	12.1	0.31

Notes: SD = standard deviation.

The T test was used for the comparison of two means. The Chi-square test was used for comparing percentages.

Supplementary Table 3. Baseline nutritional intake of the study participants according to frailty status (*N*=1,822).

	Total	Non frail	Frail	*p* Value for trend*
Energy (kcal/d), mean (SD)	2037.0 (566.4)	2048.7 (559.6)	1888.3 (630.0)	<0.001
Total protein (g/d), mean (SD)	92.0 (25.5)	92.6 (25.3)	84.0 (27.1)	<0.001
Animal protein (g/d), mean (SD)	61.1 (21.6)	61.6 (21.6)	55.3 (21.4)	0.001
Vegetal protein (g/d), mean (SD)	30.9 (10.2)	31.0 (10.2)	28.7 (10.2)	0.011
Total protein (g/kg/d), mean (SD)	1.26 (0.4)	1.27 (0.4)	1.12 (0.3)	<0.001
Animal protein (g/kg/d), mean (SD)	0.83 (0.3)	0.84 (0.3)	0.73 (0.3)	<0.001
Vegetal protein (g/kg/d), mean (SD)	0.42 (0.2)	0.43 (0.2)	0.38 (0.2)	0.004
Total fats (g/d), mean (SD)	82.2 (30.3)	82.7 (30.0)	77.0 (34.4)	0.039
Monounsaturated fatty acids (g/d), mean (SD)	36.1 (14.0)	36.3 (13.8)	33.3 (15.6)	0.018
Saturated fatty acids (g/d), mean (SD)	24.7 (11.3)	24.7 (11.1)	24.3 (12.8)	0.70
Long-chain ω-3 fatty acids (g/d), mean (SD)	0.9 (0.9)	1.0 (0.9)	0.7 (0.8)	0.002
Alpha-linolenic acid (g/d), mean (SD)	1.1 (0.7)	1.1 (0.8)	1.0 (0.6)	0.032
Linoleic acid (g/d), mean (SD)	11.7 (6.5)	11.8 (6.5)	10.7 (5.9)	0.06
Total fats (g/kg/d), mean (SD)	1.12 (0.4)	1.13 (0.4)	1.02 (0.4)	0.003
Monounsaturated fatty acids (g/kg/d), mean (SD)	0.49 (0.2)	0.50 (0.2)	0.44 (0.2)	0.001
Saturated fatty acids (g/kg/d), mean (SD)	0.34 (0.2)	0.37 (0.2)	0.32 (0.15)	0.246
Long-chain ω-3 fatty acids (g/kg/d), mean (SD)	0.013 (0.01)	0.013 (0.01)	0.010 (0.01)	<0.001
Alpha-linolenic acid (g/kg/d), mean (SD)	0.016 (0.01)	0.016 (0.01)	0.013 (0.008)	0.012
Linoleic acid (g/kg/d), mean (SD)	0.16 (0.09)	0.16 (0.09)	0.14 (0.09)	0.017
Carbohydrates (g/d), mean (SD)	213.0 (63.2)	213.6 (63.0)	205.4 (66.2)	0.15
Simple sugars (g/d), mean (SD)	92.2 (33.3)	92.4 (33.3)	89.3 (33.0)	0.30
Polysaccharides (g/d), mean (SD)	120.9 (48.5)	121.2 (48.2)	116.1 (52.6)	0.24
Carbohydrates (g/kg/d), mean (SD)	2.9 (0.9)	2.9 (0.9)	2.8 (0.9)	0.037
Simple sugars (g/kg/d), mean (SD)	1.3 (0.5)	1.3 (0.5)	1.2 (0.5)	0.112
Polysaccharides (g/kg/d), mean (SD)	1.7 (0.7)	1.7 (0.7)	1.6 (0.7)	0.092
Ethanol (g/d), mean (SD)	20.0 (20.3)	20.2 (20.4)	14.6(18.0)	0.07
Fiber (g/d), mean (SD)	24.3 (8.3)	24.5 (8.3)	21.6 (7.3)	<0.001

Notes: SD = standard deviation.

The T test was used for the comparison of two means.

Supplementary Table 4. Baseline characteristics of the study participants according to quartiles of macronutrient intake (*N*=1,822).

	Total protein		Animal protein		Vegetable protein		Monounsaturated fatty acids		Saturated fatty acids		Long-chain ω-3 fatty acids		Alpha-linolenic acid		Linoleic acid	
	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
Men (%)	48.7	48.8	48.7	48.8	48.7	48.8	48.7	48.8	48.7	48.8	48.7	48.8	48.7	48.8	48.7	48.8
Age, years (mean)	68.7	68.4	68.9	68.1	68.2	68.9*	69.2	68.8	68.8	68.9	68.0	68.2†	69.5	68.8	69.5	68.8
Level of education (%)																
Primary or less	18.6	22.2	21.1	23.7	23.0	22.0	21.5	21.5	19.5	25.7*	21.9	19.6	21.9	22.4	20.4	22.9
Secondary	25.0	24.4	23.3	24.8	26.8	23.7	22.8	25.3	23.7	22.9*	19.3	27.9	22.8	24.4	22.8	25.7
University	56.4	53.4	55.7	51.4	50.2	54.3	55.7	53.2	56.8	51.4*	58.8	52.5	55.3	53.2	56.8	51.4
Marital status (%)																
Single	8.3	5.3	7.5	5.9	7.7	8.6	7.0	5.5	7.2	7.7	7.2	7.9	6.1	7.9	6.8	7.5
Married	67.3	75.4	69.1	73.6	70.8	71.4	68.6	75.2	71.7	68.6	70.4	74.5	71.7	71.9	72.2	72.8
Divorced	4.4	4.5	4.8	2.0	4.0	4.2	3.5	2.6	4.4	3.5	4.8	2.0	2.4	2.9	2.9	4.4
Widower	20.0	17.8	18.6	18.5	17.5	15.8	20.8	16.7	16.7	20.2	17.5	15.6	19.7	17.4	18.2	15.4
Tobacco consumption (%)																
Never smoker	13.6	10.6	12.5	10.6	13.2	9.0*	11.4	10.1	9.0	14.1*	12.5	11.0	13.4	8.4	13.2	8.6
Former smoker	30.3	32.3	30.3	33.4	30.9	30.6*	30.7	32.3	29.0	29.2*	29.4	29.2	31.4	33.2	29.8	30.8
Current smoker	56.1	57.1	57.2	56.0	55.9	60.4*	57.9	57.6	62.1	56.7*	58.1	59.8	55.3	58.5	57.0	60.7
Body mass index (kg/m^2) (%)																
<25	17.1	16.5	21.1	16.5†	16.2	25.3‡	21.5	17.1	22.6	18.0*	17.3	19.3	17.3	25.7†	18.4	24.2*
25-29.9	55.3	47.7	54.0	48.6†	50.2	48.4‡	47.8	50.8	49.3	49.5*	50.2	49.2	48.7	48.8†	50.0	50.3*
≥30	27.6	35.8	25.0	35.0†	33.6	26.4‡	30.7	32.1	28.1	32.5*	32.5	31.4	34.0	25.5†	31.6	25.5*
Abdominal obesity (%)	61.4	57.1	54.6	59.8	60.3	47.0‡	54.2	60.4†	50.4	38.9‡	63.2	54.5†	63.4	48.8	59.7	50.6
Chronic respiratory disease (%)	8.8	7.5	8.3	8.6	7.7	8.8	9.2	6.6	6.8	7.0	10.1	9.0	6.4	8.1	7.0	8.8
Coronary disease (%)	1.1	1.5	0.7	1.3	1.1	1.8	1.5	2.0	1.3	2.2	1.5	0.9	1.5	1.5	1.3	1.3
Stroke (%)	0.7	1.8	0.9	1.3	0.4	1.1	0.9	0.7	1.8	1.3	0.7	0.9	1.1	1.1	1.3	0.4
Osteoarthritis/arthritis (%)	42.1	51.2†	43.6	47.0	44.7	49.7	47.4	44.4	50.2	44.6*	46.9	48.8	48.5	47.9	48.0	45.3
Cancer (%)	0.7	2.6*	0.7	3.1‡	1.8	2.2	1.8	1.5	2.6	2.4	1.8	2.2	1.5	2.4	2.4	1.3
Diabetes (%)	16.0	17.8	14.3	16.7	14.7	12.5	12.1	17.6*	14.9	18.5	16.5	15.2	15.4	13.6	13.6	15.8
Depression (%)	7.7	7.0	8.3	7.7	7.7	5.9	7.5	5.7	5.9	9.0	9.2	5.5*	7.9	8.1	6.8	8.1
Number of medications (mean)	1.9	2.0	2.0	1.9	1.8	1.9	1.9	2.1	2.0	2.1	2.1	1.8‡	1.9	2.0	2.0	1.8
Ex-drinker status (%)	10.5	10.8	11.4	8.1	5.3	10.3‡	7.5	11.0†	6.6	14.1‡	14.0	8.6†	8.6	9.7	9.0	12.3
Dietary variables																
Energy, (kcal/d) (mean)	2061.5	2014.0	2060.4	2012.6	2014.3	2018.0	2008.7	1970.8	1994.0	2031.9	2053.1	1994.5	2039.9	2048.0	1992.8	2042.3
Total protein (g/d) (mean)	74.7	111.7‡	76.3	110.3‡	92.4	92.4	88.8	90.0	90.8	92.7	85.7	98.0‡	88.8	93.4†	93.5	89.0*
Total fats (g/d) (mean)	82.3	82.2	80.4	85.1	86.7	75.2‡	66.8	93.0‡	69.6	93.5‡	82.7	81.7	74.0	88.5‡	71.7	90.2‡
Carbohydrates (g/d) (mean)	226.7	194.9‡	235.5	185.6‡	185.9	232.0‡	233.1	181.6‡	226.2	193.4‡	223.8	198.2‡	224.9	205.3‡	216.4	206.3*
Ethanol (g/d) (mean)	26.8	13.8‡	23.0	17.5†	27.8	13.5‡	29.9	13.1‡	26.1	14.2‡	19.8	19.5	29.0	16.6‡	27.7	13.8‡
Fiber (g/d) (mean)	23.2	24.6	26.2	22.3‡	18.7	30.3‡	24.4	23.4*	27.3	21.2‡	24.3	23.9	22.6	26.3‡	23.2	25.2‡

Notes: Q1 = Quartile 1 (lowest); Q4 = Quartile 4 (highest).

P for linear trend among quartiles:* p <0.05; † p <0.01; ‡ p <0.001.

The cut-off points for the quartiles (g/d) were: in men (Total protein Q1: (31.6-82.9), Q2: (83.0-98.3), Q3: (98.4-115.7), Q4: (115.8-218.4); Animal protein Q1: (10.5-51.6), Q2: (51.7-64.3), Q3: (64.4-80.1), Q4: (80.2-199.1); Vegetable protein Q1: (10.2-26.4), Q2: (26.5-32.6), Q3: (32.7-39.5), Q4: (39.6-78.5); Monounsaturated fatty acids Q1: (5.4-30.3), Q2: (30.4-39.2), Q3: (39.3-48.4), Q4: (48.5-102); Saturated fatty acids Q1: (4.6-19.1), Q2: (19.2-25.6), Q3: (25.7-33.0), Q4: (33.1-103.7); Long-chain ω-3 fatty acids Q1: (0-0.4), Q2: (0.5-0.8), Q3: (0.9-1.4), Q4 (1.5-9.9); Alpha-linolenic acid Q1: (0.2-0.7), Q2: (0.8-1.0), Q3: (1.1-1.3), Q4: (1.4-10.6); Linoleic acid Q1: (1.5-8.3), Q2: (8.4-11.5), Q3: (11.6-15.8), Q4: (15.9-59.3); in women (Total protein Q1: (26.9-68.0), Q2: (68.1-81.8), Q3: (81.9-95.6), Q4: (95.7-181.3); Animal protein Q1: (11.3-42.4), Q2: (42.5-52.8), Q3: (52.9-65.5), Q4: (65.6-199.8); Vegetable protein Q1: (5.9-21.3), Q2: (21.4-27.3), Q3: (27.4-33.4), Q4: (33.5-77.8); Monounsaturated fatty acids Q1: (4.3-23.4), Q2: (23.5-30.6), Q3 (30.7-39.9), Q4: (40.0-86.1); Saturated fatty acids Q1: (2.4-15.2), Q2: (15.3-20.0), Q3: (20.1-28.1), Q4: (28.2-87.3); Long-chain ω-3 fatty acids Q1: (0-0.3), Q2: (0.4-0.6), Q3: (0.7-1.1), Q4: (1.2-6.3); Alpha-linolenic acid Q1: (0.2-0.6), Q2: (0.7-0.8), Q3: (0.9-1.1), Q4: (1.2-6.4); Linoleic acid Q1: (1.7-6.3), Q2: (6.4-9.1), Q3: (9.2-13.4), Q4: (13.5-56.1).

Supplementary Table 5. Odds ratios (95% confidence interval) of the frailty components according to quartiles of intake of total protein, animal protein, and monounsaturated fatty acids in the Seniors-ENRICA cohort study *(N*=1,822).

	Low physical activity OR (95% CI)	Slowness OR (95% CI)	Unintentional Weight loss OR (95% CI)	Muscle weakness OR (95% CI)	Exhaustion OR (95% CI)
Total protein					
Quartile 1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	0.92 (0.63-1.34)	0.63 (0.44-0.92)*	0.92 (0.55-1.54)	1.11(0.81-1.53)	0.99 (0.64-1.51)
Quartile 3	0.80 (0.54-1.20)	0.58 (0.39-0.87)†	1.07 (0.63-1.84)	1.22 (0.87-1.70)	0.96 (0.61-1.51)
Quartile 4 (highest)	0.84 (0.56-1.27)	0.63 (0.41-0.96)*	0.79 (0.44-1.41)	1.19 (0.84-1.69)	0.74 (0.46-1.19)
p Value for trend	0.33	0.022*	0.57	0.27	0.23
Animal protein					
Quartile 1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	0.81 (0.56-1.19)	0.73 (0.51-1.04)	0.87 (0.53-1.43)	1.12 (0.82-1.54)	0.75 (0.49-1.15)
Quartile 3	0.85 (0.58-1.24)	0.54 (0.37-0.79)†	0.65 (0.38-1.11)	1.13 (0.83-1.55)	0.83 (0.55-1.27)
Quartile 4 (highest)	0.77 (0.51-1.15)	0.45 (0.30-0.69) ‡	0.67 (0.39-1.15)	1.02 (0.73-1.42)	0.62 (0.39-0.99)*
p Value for trend	0.25	<0.001‡	0.09	0.90	0.08
Monounsaturated fatty acids					
Quartile 1 (lowest)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	1.02 (0.69-1.53)	1.20 (0.80-1.79)	0.48 (0.28-0.82)†	1.53 (1.09-2.14)*	0.68 (0.42-1.08)
Quartile 3	1.13 (0.74-1.71)	0.83 (0.53-1.30)	0.40 (0.22-0.71)†	1.56 (1.10-2.22)*	0.79 (0.49-1.27)
Quartile 4 (highest)	0.93 (0.59-1.48)	0.96 (0.60-1.54)	0.45 (0.24-0.82)†	1.06 (0.72-1.55)	0.85 (0.51-1.41)
p Value for trend	0.89	0.48	0.012*	0.90	0.75

Notes: OR = odds ratio; CI = confidence interval.
The *p* values for the OR were obtained using logistic regression: * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$. Analyses adjusted as in Model 3 in Table 1.

3.4. DISCUSSION

Total protein, animal protein, and MUFA intakes were inversely associated with incident frailty in community-dwelling older adults. Moreover, higher animal protein intake was associated with lower risk of slow walking speed, and higher MUFA intake was associated with reduced risk of unintentional weight loss. These results were obtained in a sample that was slightly younger than other well-known cohorts in this field, such as the InCHIANTI study (87), and thus the incidence of frailty was somewhat lower. However, our results did not seem to vary with age.

3.4.1. Protein intake

In addition to the WHI-OS (56), the association of protein intake and frailty has been assessed in three cross-sectional analyses (88-90), with partially consistent results. In Italy, the InCHIANTI study (88) reported that the prevalence of frailty in older adults in the lowest quintile of protein intake was double that of those in the highest quintile. Likewise, in Japanese older women (89), a higher protein intake was associated with lower prevalence of frailty, regardless of the source of protein. Finally, among 194 community-dwelling elders in Germany, the distribution of protein consumption throughout the day, but not the total amount of protein intake, was significantly related to frailty (90). Frail participants showed a different and more uneven distribution of their protein intake during the day, with lower intake at breakfast and higher intake at lunch. However, given the cross-sectional design in these three analyses, frail participants may have changed their diet due to exhaustion, weight loss, or weakness.

In the Health, Aging and Body Composition (Health ABC) study (53), total and animal protein intake, but not vegetable protein, was associated with less lean mass loss in older adults. Those in the highest quintile of protein intake lost 40% less lean mass than did those in the lowest quintile. Similar results were found for appendicular lean mass, which best discriminates the presence of weakness. These results contrast with ours because we did not find an association between protein intake and weakness.

Our results are also of interest because they may shed light on the current discussion on recommended protein intake in older adults. The current RDA for protein is 0.8 g/kg/d, and the estimated average requirement is 0.66 g/kg/d. These values apply to all the healthy adult population, including older adults (91). Some studies question whether these recommendations are adequate for this population subgroup, because of the high frequency of significant sarcopenia (91). In our study, the median total protein intake in the third quartile was slightly above the RDA, corresponding to 1.28 g/kg/d (about 95 g/d). Above this value, a higher protein intake was not related to a further decrease in frailty risk. These findings concur with those of the WHI-OS (56) and the Health ABC study (53) because in both the studies, the highest quintile of protein intake was 1.20 g/kg/d, which is 50% higher than the current RDA (53, 56). Moreover, additional evidence supports the recommendation of protein intake averaging 1.20 g/kg/d (60, 61), because the threshold for anabolic intake of protein is greater in elderly participants compared with that in young adults. Finally, this amount of protein intake has not been associated with any detrimental effect in the general population (92).

In our study, no association between vegetable protein and frailty was found. This could be because the intake of vegetable protein was lower than that of animal protein, or because the latter is richer in essential amino acids (51) which, in turn, could be the primary inducer of muscle protein anabolism in older adults. Moreover, in a study among normal weight and sedentary women, animal protein was the single predictor of muscle mass index (54).

The observed inverse association between protein intake and slowness is also noteworthy, because slow gait is one of the best predictors of the adverse health outcomes associated with frailty (93). Interestingly, no association was found between protein intake and muscle weakness. Walking is a complex activity, which requires sufficient lean body mass, strength, neurological functioning, and balance, among other functions. Unfortunately, our study only assessed gait speed and strength but not the other variables required for walking.

Overall, our findings support a protein intake of at least 1.2 g/kg/d in older adults, in line with new dietary recommendations. Protein should derive from the main food sources (such as chicken, milk, white fish, matured cheese, beef, ham,

white bread), with a preference for those with lower content in saturated fats or salt (94).

3.4.2. MUFA intake

No previous epidemiologic studies have assessed the role of different types of fats on frailty, although a cross-sectional study in the United Kingdom found that a higher intake of SFAs and MUFAs was associated with a lower fat-free mass in women aged 18 to 79 years (95).

The main sources of MUFAs in Spain and other Mediterranean countries differ from those in northern Europe, America, or Asia. The main source of MUFAs in Spain is olive oil (an essential component of the Mediterranean diet), which is flavorful and often consumed with vegetables, which also have shown a beneficial effect on frailty (96). Other MUFA-rich foods frequently consumed in Spain are red meat, dairy, and pastries. By contrast, the main contributors to total MUFA intake in non-Mediterranean countries were meat and meat products, added fats, and dairy products (97).

Certain mechanisms may account for the relationship between MUFA intake and frailty. The Mediterranean diet (rich in MUFAs) has been associated with lower levels of inflammatory markers (98, 99). In addition, these markers are strongly related to frailty (100) and, to some extent, to unintentional weight loss (101). Moreover, extra-virgin olive oil is rich in tocopherols, which provide oxidative stability and have scavenging activity (102). Tocopherols and tocotrienols protect lipids from peroxidation due to their complex organization in the phospholipid bilayers, and α-tocopherol spontaneously associates with unsaturated fatty acids (103, 104).

We found a strong inverse association between MUFA intake and unintentional weight loss. Although the mechanisms of this association are uncertain, there is some evidence that a high-MUFA diet versus a high-SFA diet may help in maintaining body weight, energy balance, and insulin sensitivity (105). Moreover, fatty acids produce a lower satiating effect than the same isocaloric quantity of dietary carbohydrate (106), which could prevent unintentional weight

loss.

Overall, our results suggest that intake of MUFAs from olive oil, as consumed in the traditional Mediterranean diet, could contribute to frailty prevention.

The strengths of this study include its prospective design and the adjustment for many potential confounders. Among the limitations was the relatively small number of frailty cases, which nevertheless did not impede observation of several significant associations. Also, diet was self-reported and a certain recall bias cannot be ruled out. Lastly, although animal protein and MUFA intake were associated with incident frailty after adjustment for an extensive number of potential confounders, some residual confounding cannot be dismissed.

In conclusion, in this prospective study in older adults in Spain, a higher intake of total and animal protein and MUFAs was associated with lower risk of frailty. These findings add to the evidence base for the development of recommendations on food and nutrient intake in older adults.

4. Protein Intake and Risk of Falls: A Prospective Analysis in Older Adults

4.1. INTRODUCTION

Falls are a major public health concern for the older adult population (25-27). Several epidemiological studies have identified medical risk factors for falling among older adults, such as age, depression, polypharmacy, sleeping medications, and hypotension (38, 39). However, the prevention of falls among the elderly is complex (39), and potential dietary determinants are only poorly identified (41).

Previous results from the Framingham Study (107) showed no association between protein intake and falls. However, the Framingham Study showed a protective association among those who had lost weight. Moreover, in the MrOS (108), neither dietary protein intake nor vitamin D was associated with a decreased risk of falls. In addition, there is no conclusive information about the role of protein intake and the risk of hip fracture in older adults (109, 110).

The main objective of this study was to assess the prospective association between total protein intake and the risk of falling in a community-dwelling population of older adults from Spain. The secondary objectives were to assess the association of animal protein and vegetable protein intake with the risk of falling in the same population and to summarize the findings from the literature. Furthermore, the analysis was stratified according to participants who experienced unintentional weight loss.

4.2. METHODS

4.2.1. Study design and Population

The data were collected for the Seniors-ENRICA cohort study that focused on a cohort of noninstitutionalized individuals aged 60 and older in Spain. The cohort was derived from the ENRICA study, a survey conducted in 2008-2010 that was representative of the noninstitutionalized adult population in Spain (80, 81). ENRICA study participants 60 years or older comprised the Seniors-ENRICA cohort (*N*=3,289). At baseline, data were collected in three stages. First, a computer-assisted telephone interview was performed to obtain information on sociodemographic factors, health behaviours, and morbidity. Second, a home visit was made to collect blood and urine samples. Third, a second home visit was made

to obtain a dietary history and prescribed medications; a physical examination was also performed during this visit. Participants were followed up until 2012 (mean follow-up time of 3.5 years), when a second wave of data collection was performed. Of the initial sample, 675 participants were lost to follow-up, 95 died, 18 were excluded for a lack of data related to falls, 36 were excluded for a lack of data on potential confounders, and 1 was excluded for a lack of reliable dietary information data. The final analyses were performed with 2,464 subjects. The baseline sociodemographic, lifestyle, and clinical characteristics of the participants included in the analyses were similar to those of the participants lost to follow-up (data not shown) (80).

The study participants gave written informed consent. The Clinical Research Ethics Committee of La Paz University Hospital in Madrid approved the study.

4.2.2. Study Variables

4.2.2.1. Diet

Information on diet was collected using a computerized dietary history (HD)-ENRICA that collects habitual dietary consumption practices during the previous year with questions related to all possible meals throughout the day. The history first asks about the food eaten upon arising in the morning and ends with asking about the food consumed before going to bed. Questions were asked about the food consumed in a typical week, and all foods consumed at least once every 15 days were recorded. In addition, information on seasonal and weekend food consumption was obtained. The data were collected by certified interviewers. The protein consumption collected with this instrument was validated against seven 24-hour recalls in the preceding year, and high correlation coefficients were obtained (total protein $\rho = 0.59$; animal protein $\rho = 0.62$; vegetable protein $\rho = 0.62$) (82). The intake of total energy, total protein, animal protein, and vegetable protein, as well as the intake of caffeine, calcium, vitamin D and alcohol, was estimated using standard food composition tables from Spain (111, 112).

4.2.2.2. Falls

A fall was defined as "an event which resulted in a person coming to rest inadvertently on the ground or floor or other lower level" (113). At the end of follow-up, participants reported the number of falls experienced in the preceding year by answering the following question: “How many times did you fall during the past year?” The responses were recorded on a continuum from zero (no falls) to nine or more. Participants who had fallen were asked this question: “As a consequence of the fall, did you need to go to the doctor, go to the emergency room, or be hospitalized, or did you not need medical attention?” A participant was deemed to have required medical attention when the answer to any of the first three options was “yes”.

4.2.2.3. Other Variables

At baseline, information on sex, age, educational level, smoking status, and alcohol consumption was obtained. Weight and height were measured under standardized conditions (84, 114), and the BMI (weight in kilograms divided by height in meters squared, kg/m^2) was calculated. In addition, participants reported their physical activity during leisure activities and at home with the EPIC cohort questionnaire (84). The time devoted to each activity was transformed into metabolic equivalents (MET)-hours/week, and the MET-hours/week from the different activities were summed. Participants also self-reported the time spent sleeping during the night or during the day, as well as the time devoted to watching TV. Participants were also asked whether they had different chronic conditions, such as cardiovascular diseases, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, hip fracture, cancer, and depression requiring treatment. The number of daily medications was checked against medication containers, and participants reported whether they usually took medications for sleeping or hypertension. The quality of the diet was estimated by the Mediterranean Diet Adherence Screener (MEDAS) without considering wine consumption (115). Finally, participants reported whether they lived alone, and we inquired about their dependence in performing IADLs using the Lawton and Brody questionnaire (116).

In 2012, unintentional weight loss was self-reported. Every participant was

asked if he or she had experienced an involuntary weight loss of 4.5 kg (10 lb) or more in the preceding year, in accordance with the standardized definition developed by Fried et al (12).

4.2.3. Statistical Analysis

Logistic models were used to assess the risk of falls. Protein intake was classified into sex-specific tertiles, and the lowest tertile was used as the reference. The models were adjusted not only for socioeconomic variables, lifestyle, diseases, and nonprotein dietary components but also for other variables associated with falls in the literature (117).

Logistic models were established to adjust for sex; age (continuous); educational level (primary or less, secondary, or university); smoking status (never-smoker, ex-smoker, or smoker); alcohol intake status (never-drinker, occasional drinker, usual drinker, or ex-drinker); BMI (<25, 25-29.9, ≥30 kg/m^2); physical activity in MET-hours/week (continuous); time spent sleeping (continuous); time spent watching TV (continuous); presence of cardiovascular disease, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, history of hip fracture, cancer, and depression requiring treatment; number of medications (continuous); use of sleeping or hypertension medications; intake of energy, caffeine, calcium, and vitamin D; adherence to a Mediterranean diet (continuous); living alone; dependence in performing IADLs, and intake of animal protein (quartiles) or vegetable protein (quartiles) as appropriate. The *p* for linear trend was calculated by treating the tertiles as a continuous variable. Interactions between the association of interest with both unintentional weight loss and age were assessed using the likelihood ratio test, and models were compared with and without the interaction terms.

The adjusted predictive risk of falls according to unintentional weight loss status (no weight loss or weight loss) was plotted against the total protein intake considered as a continuous variable. Statistical significance was set at a two-sided $p < 0.05$. The analyses were conducted with Stata/SE, version 14.1 (StataCorp, College Station, TX).

4.3. RESULTS

The final sample comprised 2,464 participants (53.0% female) with a mean age of 69 years at baseline. After a mean follow-up of 3.5 years, a total of 522 participants (21.2%) had fallen at least once in the preceding year. The means for protein consumption were as follows: 92 g/d (SD: 26 g/d) for total protein, 61 g/d (SD: 22 g/d) for animal protein, and 31 g/d (SD: 10 g/d) for vegetable protein.

Participants consuming more total protein and animal protein were younger, less frequently ex-drinkers, heavier, taller, and more frequently obese; suffered from cancer more frequently; had higher consumption of energy, calcium, and vitamin D; followed a Mediterranean diet more frequently, lived alone less frequently; and had less dependence in performing IADLs than those consuming less total protein and animal protein. However, those who consumed more vegetable protein had similar ages, weighed less, were more physically active, had cancer less frequently, and did not consume a greater amount of vitamin D than those who consumed less vegetable protein (Table 3).

Supplementary Table 6 presents the crude results for the risk of falling. The adjusted ORs (95% CI) of falling for the three increasing tertiles of total protein intake were 1.00, 0.86 (0.66-1.11), and 0.93 (0.70-1.24); *p* for trend = 0.14. Thus when the association between total protein intake and having experienced at least one fall was assessed, the estimates were less than 1 in the fully adjusted model, but none of the associations were statistically significant. Similar results were obtained for animal and vegetable protein intake.

However, a statistically significant interaction was observed with unintentional weight loss when the association between protein intake and risk of falls was assessed; *p* for interaction = 0.004. For participants who lost 4.5 kg or more in the preceding year, the adjusted ORs (95% CI) of falling for the three increasing tertiles of total protein intake were 1.00, 0.68 (0.21-2.23), and 0.23 (0.05-1.08); *p* for trend = 0.01 (Table 4). In all cases, the estimators decreased linearly, but the interactions were statistically significant only for vegetable protein intake when those in the highest consumption tertile were compared with those in the lowest consumption tertile (OR: 0.20; 95% CI: 0.05-0.84). The interaction was statistically

significant in all analyses (Table 4 and Figure 1).

We performed sensitivity analyses to assess the robustness of the main results. We obtained similar results when excluding participants with a history of hip fracture, when reestimating the study association using different methodologies to adjust for energy consumption, when adjusting for ethanol (g/d), and when excluding Mediterranean diet as a confounder. When falls requiring medical attention were used as an end point, the results exhibited a trend similar to those of the other analyses, but the associations were not statistically significant due to a lack of events. Finally, we did not find a statistically significant interaction between protein intake and age.

Table 3. Baseline characteristics of the study participants according to tertiles of total protein, animal protein, and vegetable protein intake in the Seniors-ENRICA cohort study (*N*=2,464).

	Total protein intake				Animal protein intake				Vegetable protein intake			
	T1	T2	T3	*p* Value for trend	T1	T2	T3	*p* Value for trend	T1	T2	T3	*p* Value for trend
Age, years (mean, SD)	69.5 (6.7)	68.5 (6.4)	67.9 (5.9)	<0.001	69.3 (6.6)	68.6 (6.5)	68.0 (5.9)	<0.001	68.7 (6.5)	68.6 (6.2)	68.6 (6.4)	0.71
Educational level (%)												
Primary or less	56.3	53.9	53.7	0.48	55.7	55.6	52.6	0.57	52.1	54.0	57.8	0.19
Secondary	22.1	25.8	24.6		22.9	24.5	25.2		25.4	24.0	23.2	
University	21.5	20.3	21.7		21.4	20.0	22.2		22.5	22.0	19.0	
Smoking status (%)												
Never smoker	59.5	59.5	57.4	0.69	60.6	57.8	58.1	0.79	57.7	58.8	60.0	0.48
Ex-smoker	28.8	30.3	30.5		28.5	30.5	30.6		29.6	29.9	30.1	
Smoker	11.7	10.2	12.1		10.9	11.7	11.3		12.8	11.3	9.9	
Alcohol intake status (%)												
Never drinker	33.7	34.6	38.2	0.05	34.4	34.1	37.9	0.13	36.7	33.6	36.1	<0.001
Occasional drinker	9.3	7.9	7.4		9.5	8.5	6.6		5.6	9.9	9.6	
Usual drinker	46.6	49.3	47.9		47.2	48.1	48.5		46.0	48.1	49.7	
Ex-drinker	10.5	8.3	6.5		8.9	9.4	7.0		11.7	8.5	5.0	
Weight, kg (mean, SD)	73.0 (12.4)	73.5 (13.1)	76.1 (13.4)	<0.001	72.5 (12.4)	74.0 (12.8)	76.1 (13.6)	<0.001	74.9 (13.4)	74.3 (12.8)	73.3 (12.8)	0.009
Height, cm (mean, SD)	160.3 (8.8)	161.3 (9.2)	162.0 (8.9)	<0.001	160.6 (8.8)	161.1 (8.8)	161.8 (9.3)	0.005	160.8 (9.4)	161.5 (8.8)	161.3 (8.7)	0.22
Body mass index (kg/m^2)												
<25	18.0	22.6	17.4	0.005	21.4	18.5	18.2	0.003	15.8	20.2	22.1	0.003
25-29.9	52.0	47.9	47.1		51.7	49.4	45.9		48.4	49.3	49.3	
≥30	30.1	29.4	35.5		26.9	32.1	36.0		35.8	30.5	28.7	
Physical activity, MET (mean, SD)	57.5 (34.0)	61.0 (34.3)	60.2 (34.4)	0.11	59.2 (34.2)	60.2 (35.0)	59.2 (34.6)	0.94	55.6 (33.1)	61.2 (35.4)	61.9 (34.9)	<0.001
Time sleeping, hours (mean, SD)	7.2 (1.6)	7.1 (1.6)	7.2 (1.5)	0.53	7.1 (1.5)	7.2 (1.5)	7.1 (1.5)	0.99	7.1 (1.5)	7.2 (1.4)	7.1 (1.5)	0.81
Time watching TV, hours (mean, SD)	2.6 (1.7)	17.8 (10.9)	2.6 (1.4)	0.89	2.6 (1.7)	17.8 (10.6)	2.6 (1.6)	0.69	2.6 (1.7)	17.9 (10.9)	2.6 (1.4)	0.99
Cardiovascular disease (%)	5.2	5.5	5.7	0.91	5.7	5.7	5.0	0.76	5.0	6.3	5.1	0.42
Hypertension (%)	65.2	63.9	65.5	0.76	65.3	63.8	65.5	0.71	66.8	64.5	63.3	0.32
Diabetes (%)	16.4	13.9	16.5	0.98	14.7	16.6	15.5	0.59	15.9	16.3	14.5	0.57
Chronic pulmonary disease (%)	7.5	6.8	7.3	0.84	8.0	6.3	7.3	0.41	7.5	6.2	7.9	0.37
Arthritis or osteoarthritis (%)	46.2	49.8	50.0	0.23	47.0	49.8	49.2	0.48	47.6	48.1	50.4	0.48
History of hip fracture (%)	1.7	1.0	1.5	0.67	1.7	1.5	1.0	0.21	1.8	0.6	1.7	0.83
Cancer (%)	1.2	1.7	2.9	0.01	1.1	2.1	2.7	0.02	2.0	2.0	2.0	0.99
Depression requiring treatment (%)	8.6	8.3	7.9	0.87	8.9	7.9	8.1	0.74	8.5	8.3	8.1	0.94
Number of medications (mean, SD)	2.1 (1.9)	1.9 (1.9)	2.0 (2.0)	0.36	2.0 (1.9)	2.0 (1.9)	2.0 (2.0)	0.55	2.0 (1.9)	1.9 (1.9)	2.0 (2.0)	0.60
Sleeping treatment (%)	20.1	20.9	19.5	0.77	21.6	19.2	19.6	0.42	21.4	19.2	19.8	0.52
Hypertension treatment (%)	38.6	38.8	37.2	0.77	39.1	37.8	37.7	0.82	40.1	37.7	36.7	0.34
Energy, kcal/day (mean, SD)	1843 (1036)	2053 (514)	2262 (560)	<0.001	1963 (1039)	2033 (549)	2162 (582)	<0.001	1861 (1060)	2045 (519)	2252 (521)	<0.001
Caffeine intake, mg/d (mean, SD)	69.0 (119)	74.7 (110)	70.1 (103)	0.84	70.1 (123)	73.2 (123)	70.5 (101)	0.96	69.5 (114)	74.5 (110)	69.8 (110)	0.96
Calcium intake, mg/d (mean, SD)	714 (244)	891.4 (254)	1058 (428)	<0.001	729 (237)	884.6 (261)	1049 (439)	<0.001	872 (441)	884.4 (307)	907 (279)	0.04
Vitamin D, μ/d (mean, SD)	2.4 (2.2)	3.4 (2.5)	4.6 (3.9)	<0.001	2.5 (2.0)	3.4 (2.6)	4.5 (4.1)	<0.001	3.4 (3.7)	3.4 (2.8)	3.5 (2.7)	0.53
Mediterranean diet, MEDAS (mean, SD)	6.6 (1.7)	7.0 (1.8)	7.2 (1.7)	<0.001	6.7 (1.9)	6.9 (1.7)	7.1 (1.6)	<0.001	6.7 (1.5)	6.9 (1.7)	7.3 (1.9)	<0.001
Living alone (%)	20.2	15.1	14.8	0.004	19.8	13.3	17.0	0.002	19.2	17.5	13.3	0.004
Dependence in performing IADLs (%)	13.1	8.5	10.0	0.008	12.4	9.7	9.5	0.10	11.9	10.0	9.8	0.29

Notes: SD = standard deviation; METs = metabolic equivalents; MEDAS = Mediterranean Diet Adherence Screener without alcohol intake; IADLs = instrumental activities of daily living; T1 = Tertile 1 (lowest); T3 = Tertile 3 (highest).

The cut-off points for the protein intake tertiles (in g/d): for men (Total protein: T1: (18.4-87.9), T2: (88.0-108.5), T3: (108.6-284.7); Animal protein: T1: (10.3-55.7), T2: (55.8-74.5), T3: (74.6-260.2); Vegetable protein: T1: (8.1-28.5), T2: (28.6-37.1), T3: (37.2-79)); for women (Total protein: T1: (26.8-72.8), T2: (72.9-90.7), T3: (90.8-207.1); Animal protein: T1: (11.3-45.7), T2: (45.8-61.2), T3: (61.3-374.5); Vegetable protein: T1: (1.6-23.4), T2: (23.5-31.1), T3: (31.2-88.4)).

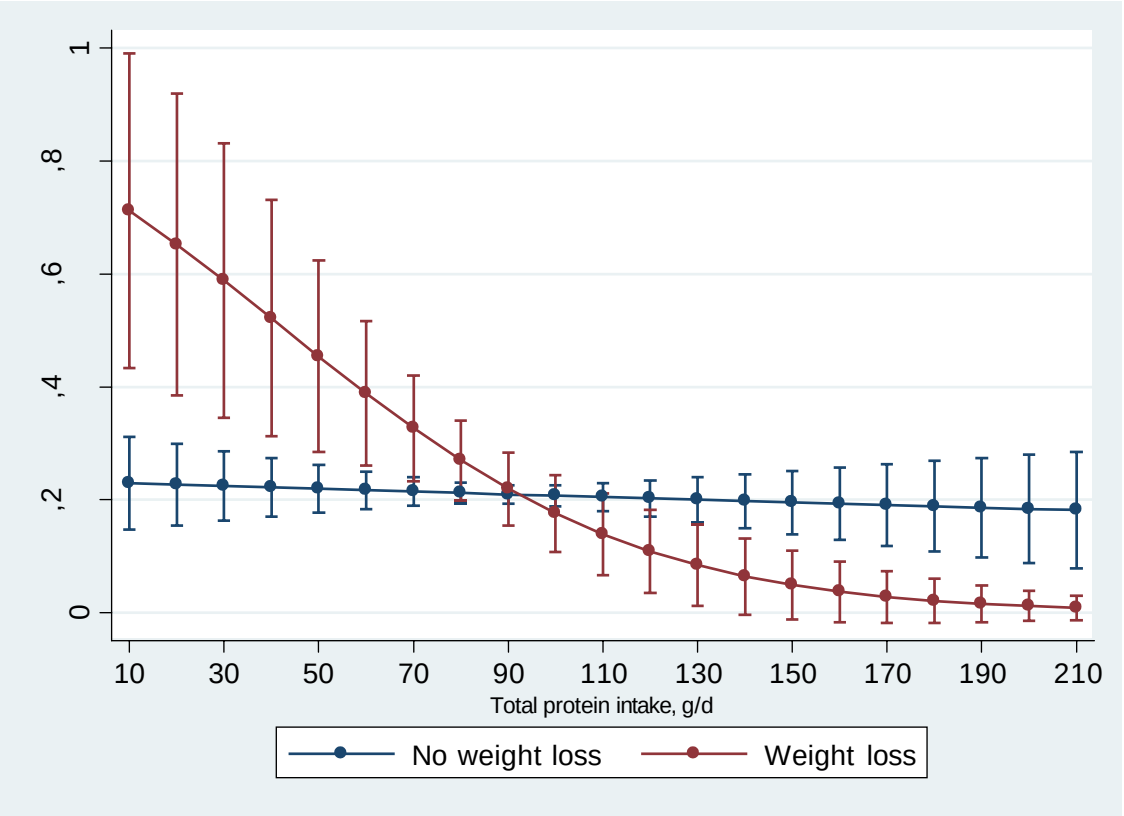
Table 4. Odds ratios (95% confidence interval) for the risk of falls according to tertiles of protein intake in the Seniors-ENRICA cohort study (*N*=2,464). Analyses were stratified by a weight loss of 4.5 kg or more in the preceding year.

	All participants		Participants without weight loss		Participants with weight loss (≥4.5 kg)		*p* Value for interaction
	Events/n 522/2464	OR (95% CI)	Events/n 477/2301	OR (95% CI)	Events/n 45/163	OR (95% CI)	
Total protein							0.004
Tertile 1 (lowest)	184/822	1 (Ref.)	164/767	1 (Ref.)	20/55	1 (Ref.)	
Tertile 2	164/822	0.86 (0.66-1.11)	147/762	0.86 (0.66-1.23)	17/60	0.68 (0.21-2.23)	
Tertile 3 (highest)	174/820	0.93 (0.70-1.24)	166/772	0.98 (0.73-1.31)	8/48	0.23 (0.05-1.08)	
p Value for trend		0.14		0.35		0.01	
Animal protein							0.017
Tertile 1 (lowest)	189/822	1 (Ref.)	169/765	1 (Ref.)	20/57	1 (Ref.)	
Tertile 2	156/822	0.81 (0.62-1.06)	140/760	0.81 (0.62-1.06)	16/62	0.65 (0.19-2.24)	
Tertile 3 (highest)	177/820	0.96 (0.71-1.28)	168/776	0.96 (0.72-1.28)	9/44	0.29 (0.07-1.28)	
p Value for trend		0.13		0.24		0.02	
Vegetable protein							0.016
Tertile 1 (lowest)	190/822	1 (Ref.)	169/772	1 (Ref.)	21/50	1 (Ref.)	
Tertile 2	160/822	0.86 (0.66-1.12)	149/771	0.86 (0.66-1.12)	11/51	0.20 (0.05-0.74)	
Tertile 3 (highest)	172/820	0.91 (0.70-1.20)	159/758	0.91 (0.70-1.20)	13/62	0.20 (0.05-0.84)	
p Value for trend		0.38		0.68		0.02	

Notes: OR = odds ratio; CI = confidence interval.

Models were adjusted for sex, age, educational level, smoking status, alcohol intake status, body mass index, physical activity, time sleeping, time watching TV, cardiovascular disease, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, history of hip fracture, cancer, depression requiring treatment, number of medications, sleeping treatment, hypertension treatment, energy intake, caffeine intake, calcium intake, vitamin D intake, Mediterranean diet, living alone, and dependence in performing instrumental activities of daily living. Animal protein and vegetable protein intake were adjusted for each other as appropriate.

Figure 1. Adjusted predictive risk of falls with 95% confidence interval according to an unintentional weight loss of 4.5 kg or more in the preceding year. The Seniors-ENRICA cohort study (*N*=2,464).



Notes: CI = confidence interval.

Models were adjusted for sex, age, educational level, smoking status, alcohol intake status, body mass index, physical activity, time sleeping, time watching TV, cardiovascular disease, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, history of hip fracture, cancer, depression requiring treatment, number of medications, sleeping treatment, hypertension treatment, energy intake, caffeine intake, calcium intake, vitamin D intake, Mediterranean diet, living alone, and dependence in performing instrumental activities of daily living.

Supplementary Table 6. Unadjusted odds ratios (95% confidence interval) for the risk of falls according to tertiles of protein intake in the Seniors-ENRICA cohort study (*N*=2,464). Analyses were stratified by a weight loss of 4.5 kg or more in the preceding year.

	All participants		Participants without weight loss		Participants with weight loss (4.5 kg or more)		*p* Value for interaction
	Events/n 522/2464	OR (95% CI)	Events/n 477/2301	OR (95% CI)	Events/n 45/163	OR (95% CI)	
Total protein							0.06
Tertile 1 (lowest)	184/822	1 (Ref.)	164/767	1 (Ref.)	20/55	1 (Ref.)	
Tertile 2	164/822	0.86 (0.66-1.11)	147/762	0.88 (0.68-1.13)	17/60	0.69 (0.31-1.52)	
Tertile 3 (highest)	174/820	0.93 (0.70-1.24)	166/772	1.01 (0.79-1.28)	8/48	0.35 (0.14-0.89)	
p Value for trend		0.14		0.95		0.03	
Animal protein							0.24
Tertile 1 (lowest)	189/822	1 (Ref.)	169/765	1 (Ref.)	20/57	1 (Ref.)	
Tertile 2	156/822	0.78 (0.62-1.00)	140/760	0.80 (0.62-1.02)	16/62	0.64 (0.29-1.41)	
Tertile 3 (highest)	177/820	0.92 (0.73-1.16)	168/776	0.97 (0.76-1.24)	9/44	0.48 (0.19-1.18)	
p Value for trend		0.49		0.84		0.10	
Vegetable protein							0.12
Tertile 1 (lowest)	190/822	1 (Ref.)	169/772	1 (Ref.)	21/50	1 (Ref.)	
Tertile 2	160/822	0.80 (0.63-1.02)	149/771	0.85 (0.67-1.09)	11/51	0.38 (0.16-0.91)	
Tertile 3 (highest)	172/820	0.88 (0.70-1.11)	159/758	0.95 (0.74-1.21)	13/62	0.37 (0.16-0.84)	
p Value for trend		0.29		0.65		0.02	

Notes: OR = odds ratio; CI = confidence interval.

4.4. DISCUSSION

No protective association was found between protein intake and the risk of falling in older adults in this community-dwelling cohort of older adults. However, when the association between protein intake and fall risk was assessed, a statistically significant interaction was observed with unintentional weight loss. An inverse trend was observed between protein intake and the risk of falls in participants who had a weight loss of 4.5 kg or more in the preceding year.

The association between protein intake and falls has been little studied. However, a cross-sectional study conducted with 100 healthy volunteers found that the protein intake of fallers and nonfallers was similar (118).

Concerning longitudinal studies, our results are in line with those from the Framingham Study (107) that included 807 participants with a mean age of 75 years, in showing no association between protein intake and falls. However, in the Framingham Study, the results for the participants who had lost weight were statistically significant and more extreme than the results in our study. Compared with our sample, the sample in the Framingham Study was slightly older and had a lower protein intake.

In the MrOS study (108), neither dietary protein intake nor vitamin D intake was associated with falls. This study comprised more than 4,000 very old postmenopausal women with a relatively low protein intake. Furthermore, neither dietary protein intake nor vitamin D was associated with falls after adjusting for potential confounders.

Hip fracture was used as the outcome in two cohort studies (109, 110). In both studies, the number of events was small, and the estimates were less than 1 but not statistically significant. However, these results are not totally comparable with those in our study. Higher protein intake has been related to an increase in urinary calcium that may lead to osteoporosis (119). Additionally, other studies have found that higher protein intake is associated with an increased intestinal absorption of calcium (120), suggesting the higher urinary calcium observed indicates calcium absorption and not bone resorption. Associations of protein intake with falls could

have determinants different from those for associations of protein intake with fractures, thus requiring further studies with a larger sample size.

The association of protein intake with falls among individuals with unintentional weight loss could be related to frailty. Frailty is an age-associated syndrome that includes sarcopenia as its pathophysiological basis (15), and unintentional weight loss, evaluated as the involuntary loss of 4.5 kg or more in the preceding year, is one of the frailty criteria in the definition developed by Fried et al (12). Another frailty criterion is muscle weakness, defined as classification in the lowest quintile of grip strength within groups based on sex and BMI in our cohort. In addition, the daily consumption of at least 1 g/kg/d protein is associated with a decreased loss of knee strength that might be an additional mechanism by which protein intake protects against falls (121). Evidence suggests that interventions to improve dietary protein intake may prevent the development of sarcopenia and frailty (56, 122). Furthermore, a systematic review and meta-analysis associated frailty with the risk of falls (123), and protein intake was related to a lower risk of frailty in the WHI-OS study (56). Additionally, in a previous analysis of this cohort (122), the intake of protein and MUFAs was strongly and inversely associated with frailty and more specifically with slow walking, considered a predictor of falls (124). Similarly, Rahi et al found that a daily protein intake of 1 g/kg/d was associated with a lower prevalence of frailty in community-dwelling older adults (125).

In the NuAge study (126), a protein intake of more than 1.0 g/kg/d was found to protect against a 1-year weight loss of 5% or more. Additionally, protein intake may attenuate not only the age-related loss of skeletal muscle but also the development of sarcopenia (53), an independent predictor of incident falls (127, 128). Interestingly, in participants in the Health ABC study who lost weight, higher protein intake was related to lower lean muscle loss, whereas in those with a stable weight, no association was found (53). Similarly, some studies related unintentional weight loss to an increased risk of frailty fractures and falls (129, 130). Thus, two of the frailty criteria, namely unintentional weight loss and muscle strength, were associated with protein intake. Moreover, frailty was associated with the risk of falls (123).

Finally, laboratory analyses have shown that some proteins may influence unintentional weight loss in older humans; some of these proteins (such as neuropeptide Y and peptide YY) are altered in both ageing animals and ageing humans (131). Therefore, our results are consistent with existing biological and medical knowledge and emphasize the important role of dietary protein intake on sarcopenia prevention and muscle protein synthesis (132) mediated or not mediated by frailty.

Some authors suggest increasing the recommendations for the dietary allowance of protein that currently is 0.8 g/kg/d (91). Additionally, a randomized controlled trial performed with malnourished older adults showed that an intervention consisting of a protein-enriched diet, calcium vitamin supplements, and telephone counseling by a dietitian decreased the number of patients who suffered falls (133), emphasizing the protective effect of protein consumption in malnourished older adults.

Our results may be relevant in older adults because protein intake might decrease the risk of frailty and falls among individuals who lose weight. Of special interest are proteins from vegetables, since the consumption of fruit and vegetables has also been associated with a reduced risk of frailty (134), and fruit and vegetables seem to be especially beneficial for individuals who have lost weight.

A strength of this study is its prospective design. Additionally, the study results were robust because they did not change substantially across many sensitivity analyses. A relatively large number of events were included, and participants were an unselected sample of free-living community residents. However, limitations of the study should be noted. As in most nutritional epidemiology studies, diet was self-reported. The HD-ENRICA data were validated against seven 24-hour recalls collected every 2 months during the previous year (82). However, recall bias cannot be totally ruled out when questionnaires are used to assess habitual dietary consumption. Additionally, the underreporting of falls is possible because of recall bias, thus leading to a misclassification bias toward the null value. Additionally, residual confounding cannot be ruled out because of the observational design. Finally, a survival bias could be present because hip fracture increases mortality, and participants lost to follow-up could be more likely to have

experienced weight loss and falls. However, the effect of this bias is probably small because hip fracture occurs in only approximately 1% of falls (135), and the baseline characteristics of the participants included in the analyses were similar to those of the participants lost to follow-up.

In conclusion, no protective association was found between protein intake and the risk of falling in older adults. However, participants who had experienced an unintentional weight loss of 4.5 kg or more in the preceding year benefited substantially from high protein intake. Consequently, a broad and integrative approach is needed to address the association between protein intake and fall prevention in older adults. Further studies should consider the influence of weight loss on this association.

5. Ultra-processed Food Consumption and Incident Frailty: A Prospective Cohort Study of Older Adults

5.1. INTRODUCTION

The frailty syndrome is characterized by a loss of functional reserve, and sarcopenia is its most important pathophysiological substrate. Frail older adults have an increased vulnerability even to minor stressors (such as having a fever or a urinary tract infection), and it has been linked to a rise in institutionalization and premature death (12, 15, 136). Frailty is a potentially reversible syndrome through physical activity and a high quality diet.

Dietary factors have been related to frailty and disability (48, 137). Total protein, animal protein, and MUFA intake is inversely associated with the risk of frailty (122). Also, the intake of antioxidants, B-vitamins (138-140), and the adherence to a Mediterranean dietary pattern (141, 142) have been related to a lower incidence of frailty. On the other hand, mixed results have been obtained for the consumption of dairy products (143).

Ultra-processed foods are defined as food or drink products formulated mostly or entirely from substances derived from foods and additives, with little if any unaltered food (66, 70). These foods include extracted or refined oils, hydrogenated oils and fats, protein isolates, different types of flour and starches, sugar variants, and remnants of animal foods (63-65). They usually have a high content of total fat, saturated fat, trans fat, simple sugars, and salt, but a low content of proteins, fiber, and vitamin density (66-69).

Ultra-processed foods are durable, palatable, ready to eat, drink, or heat, and mostly affordable (64, 68). After aggressive marketing (144), their consumption is growing substantially even in middle- and low- income countries (63). There is evidence that ultra-processed food consumption is associated with an increased risk of chronic diseases and metabolic risk factors, such as overweight/obesity (73, 74), hypertension (72), high total cholesterol and low-density lipoprotein cholesterol levels in children (75), metabolic syndrome in adolescences (76), cancer (77), and other chronic diseases (78).

Although a recent paper analyzing data from the Senior-ENRICA cohort found

that the consumption of added sugars in the diet of older people was associated with frailty, and mainly when present in processed foods (145), to our knowledge, no previous epidemiological study have comprehensively assessed the association of ultra-processed food intake with frailty.

5.2. METHODS

5.2.1. Study Design and Population

Data were taken from the Seniors-ENRICA cohort. In brief, the cohort was established in 2008-2010 with 2,614 individuals of the community-dwelling population aged 60 years and older in Spain (81, 141). At baseline, a computer-assisted phone interview was performed to obtain information on sociodemographic factors, lifestyle, and morbidity. Additionally, two home visits were performed to collect blood and urine samples, to obtain a dietary history, and to conduct a physical examination. Participants were followed up until 2012 (mean follow-up time of 3.5 years), when a second wave of data collection was performed. From the initial sample, 434 participants were lost, and 95 died during follow-up. Among survivors, 174 were excluded for being frail or a lack of data on frailty at baseline, 81 were excluded for a lack of data on potential confounders, and 8 were excluded for a lack of reliable dietary information data. Finally, analyses were performed with 1,822 participants. The sociodemographic, lifestyle, and clinical characteristics at baseline were similar in those who provided updated information in 2012 and those who did not (data not shown).

The study participants gave written informed consent. The Clinical Research Ethics Committee of "La Paz" University Hospital in Madrid (Spain) approved both, the baseline and follow-up studies.

5.2.2. Study Variables

5.2.2.1. Diet

Habitual food consumption was assessed with a validated computerized face-to-face DH-ENRICA developed from that used in the EPIC cohort study in Spain (82, 146). The dietary history included all habitual foods consumed at least once every 15 days. During the interview, the information was summarized in a typical week

representing the previous year. DH-ENRICA allowed registering a wide amount of food items and 30 different cooking methods, and used a set of more than 120 photographs to help in estimating the amount of consumption.

All recorded foods were classified according to NOVA based on the extent and purpose of industrial food processing (66). The justification of NOVA food definition and classification has been reported elsewhere (67, 70, 147). In brief, the NOVA classification includes four groups: (i) unprocessed or minimally processed foods: fruit and vegetables, grains, nuts and seeds, fresh and pasteurized milk, and natural yogurt with no added sugars or artificial sweeteners; (ii) processed culinary ingredients: salt, sugar, honey, vegetable oils, butter, lard, and vinegar; (iii) processed foods: canned or bottled vegetables and legumes, fruit in syrup, canned fish, unpackaged cheeses, freshly made bread, and salted nuts and seeds; and (iv) ultra-processed foods. Ultra-processed foods are those formulated mostly or entirely from food-derived substances. Some examples of ultra-processed foods are burgers, frozen pizza and pasta dishes, nuggets and fish fingers, crisps, mass-manufactured breads and cakes, cookies, jams and confectionery, margarines, cereal bars, carbonated and other sugary drinks, sugary or sweetened milks, and various snack products (63-65, 71, 72). Food items according to NOVA classification are shown in Supplementary Table 7. The intake of total energy from ultra-processed foods was estimated using standard food composition tables from Spain (82).

5.2.2.2. Frailty

A slight modification of the frailty definition proposed by Fried et al in the Cardiovascular Health Study was used (12). Specifically, frailty was defined as having three or more of the following five phenotypic criteria as follows: (i) low physical activity, defined as walking 2.5 hours per week or less in men and 2 hours per week or less in women (corresponding to the lowest quintiles in our cohort); (ii) slowness, considered as the lowest quintile in our study sample for the 3-meter walking speed test, adjusted for sex and height; in men, the cut-off points were 0.47 and 0.45 m/second in those with height ≤173 cm and >173, respectively; corresponding values in women were 0.37 and 0.40 m/second; (iii) unintentional weight loss, when 4.5 kg (10 lb) or more of body weight was lost in the preceding year; (iv) muscle weakness, defined as

the lowest quintile of maximum grip strength on the dominant hand, adjusted for sex and BMI in our cohort. Strength was measured with a Jamar dynamometer, and we selected the highest value in two consecutive measurements; in men, the cut-off points were <29, <30 and <32 kg, respectively, in those with BMI ≤24, 24.1-28, and >28 kg/m^2; the corresponding values in women were <17, <17.3, <18, and <21 kg, respectively, in those with BMI ≤23, 23.1-26, 26.1-29, and >29 kg/m^2; (v) exhaustion, evaluated as responding to "3 or more days a week" to at least one of the following questions taken from the Center for Epidemiologic Studies-Depression scale (83): "I felt that anything I did was a big effort" or "I felt that I could not keep on doing things."

5.2.2.3. Other Variables

At baseline, data on sociodemographics, lifestyles, and morbidities were collected. Self-reported information was obtained on sex, age, marital status, educational level, tobacco consumption, ex-drinker status, and number of medications used (checked against packages). Weight, height, and WC were measured at home under standardized conditions, and BMI, weight in kilograms divided by square height in meters, was calculated (114). Information on physical activity, in the household and during leisure time, was collected with the questionnaire used in the EPIC-Spain cohort (84). Study participants also reported if they had suffered from any of the following physician-diagnosed prevalent conditions: chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, and depression requiring treatment.

5.2.3. Statistical Analysis

The association between ultra-processed food intake and the risk of frailty was summarized with ORs (95% CI) obtained from logistic regression. The percentage of total energy intake ingested from ultra-processed foods was classified into sex-specific quartiles and the lowest quartile was used as reference. Considering the percentage of total energy intake from ultra-processed foods we take into account the high energy density of these foods. We replicated the same analyses expressing ultra-processed food consumption in gram per day/kg of body weight (g/kg), to consider the body size, as well as the ultra-processed food intake with low energy contribution. The *p* for linear trend was calculated by using quartiles as a continuous variable.

Sociodemographic, lifestyle, and morbidity variables associated with frailty in the literature, were considered as potential confounders. Thus, three logistic models were built with progressive levels of adjustment: the first one was adjusted for sex and age (60–69, 70–79, 80 years and older); the second one additionally adjusted for educational level (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (current, former, never smoker), and ex-drinker status; Model 3 was also adjusted for chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, depression requiring treatment, and number of medications. Among robust participants at baseline, we also tested the association between ultra-processed food intake and each frailty criterion, using the same type of modeling.

We assessed the ultra-processed food intake by food groups, expressed as a percentage of total energy. Food groups were classified into sex-specific quartiles except when: a) the intake of ultra-processed foods from a food group occurred in less than 50% of the participants, in which variables were classified into sex-specific tertiles; b) when it occurred in less than 25% of the participants, the ORs (95% CI) were calculated between participants who consumed the food group compared with those who did not.

Statistical significance was set at two-sided p <0.05. The analyses were performed with Stata/SE, version 14.1 (StataCorp, College Station, TX).

5.3. RESULTS

The mean age of the 1,822 participants was 68.7, and 51.3% were women. On average, the contribution of ultra-processed food intake to total energy intake was 19.3% (from 0% to 79.7%). We also estimated total energy intake across increasing quartiles of ultra-processed food consumption, and a wide variation was found in the relative contribution of each NOVA category to total energy intake among quartiles of ultra-processed food intake (Supplementary Figure 1). Given that energy intake from these foods was lower in men (17.7%) than in women (20.7%), the exposure variables were modeled as sex-specific quartiles. Compared with participants in the lowest quartile of ultra-processed food intake, those in the highest quartile consumed more total energy, were more often ex-drinkers, had depression more frequently, but suffered

from osteoarthritis/arthritis less frequently (Table 5).

Over a mean follow-up of 3.5 years, 132 cases of frailty were ascertained. In the fully adjusted analyses, the ORs (95% CI) of frailty risk across quartiles of ultra-processed food intake, expressed as a percentage of total energy, with respect to the first quartile were 1.52 (0.78-2.96), 2.98 (1.62-5.50), and 3.67 (2.00-6.73); *p* for trend = <0.001). The corresponding ORs (95% CI) when ultra-processed foods were expressed in g/kg were 2.42 (1.32-4.41), 2.48 (1.37-4.49), and 2.57 (1.41-4.70); *p* for trend = 0.004 (Table 6, Model 3).

When each frailty criterion was considered, the greatest OR (95% CI) was the one for unintentional weight loss, which was 3.33 (1.86-5.97) for the highest versus the lowest quartile of ultra-processed food intake, expressed as a percentage of total energy; *p* for trend = <0.001. Results had the same direction for other frailty components (low physical activity and slowness), and were also consistent when ultra-processed food intake was measured in g/kg (Table 7).

Main food groups contributing to ultra-processed food intake were meat and meat products (17.8%), cakes and pastries (12.4%), cookies (11.9%), yogurts and fermented milks (9.5%), jams and confectionary (8.9%), and pre-cooked dishes (7.4%). The highest versus the lowest tertiles of non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks), yogurts and fermented milks (natural and non-sugary yogurts are not included in this category), as well as cakes and pastries were significantly associated with incident frailty. For cookies, dairy desserts, meat and meat products, sauces and dressings, pre-cooked dishes, and soft drinks the associations were in the same direction, but no statistical significance was achieved (Figure 2).

Similar results were obtained in sensitivity analyses that removed unintentional weight loss from the frailty criteria, when age was expressed as a continuous variable, and when physical activity and total energy intake were considered as potential confounders.

Table 5. Baseline characteristics of the cohort participants according to quartiles of ultra-processed food consumption as a percentage of total energy (% energy), in the Seniors-ENRICA cohort study (*N*=1,822).

	Ultra-processed food consumption (% energy)				
	Q1 (lowest) (n = 456)	Q2 (n = 455)	Q3 (n = 456)	Q4 (highest) (n = 455)	*p* Value for trend
Total energy (kcal/day), mean ± SD	1903 ± 525	2003 ± 562	2040 ± 529	22201 ± 606	<0.001
Ultra-processed food consumption (% energy), mean ± SD	6.5 ± 3.1	14.1 ± 2.5	21.7 ± 3.1	34.9 ± 7.7	<0.001
Ultra-processed food consumption (g per day), mean ± SD	116 ± 88	216 ± 133	294 ± 157	417 ± 192	<0.001
Weight (kg), mean ± SD	73.3 ± 12.6	74.7 ± 12.6	74.0 ± 13.0	75.2 ± 13.0	0.067
Ultra-processed food consumption (g/kg), mean ± SD	1.6 ± 1.2	3.0 ± 1.9	4.1 ± 2.2	5.7 ± 2.7	<0.001
Age (years), mean ± SD	68.9 ± 6.2	68.6 ± 6.1	68.6 ± 6.5	68.6 ± 6.6	0.539
Educational level, (%)					0.290
Primary or less	21.7%	20.4%	21.1%	22.7%	
Secondary	24.1%	24.6%	22.4%	28.6%	
University	54.2%	55.0%	56.6%	48.8%	
Marital status, (%)					0.068
Single	7.7%	7.0%	5.7%	6.8%	
Married	74.1%	73.2%	73.5%	69.7%	
Divorced	2.4%	3.1%	3.1%	4.4%	
Widower	15.8%	16.7%	17.8%	19.1%	
Tobacco consumption, (%)					0.102
Current smoker	10.3%	9.0%	14.9%	12.1%	
Former smoker	31.6%	30.8%	28.5%	32.8%	
Never smoker	58.1%	60.2%	56.6%	55.2%	
Ex-drinker status, (%)	7.2%	9.2%	9.0%	13.0%	0.006
Chronic conditions, (%)					
Chronic respiratory disease	7.5%	8.1%	7.9%	6.8%	0.697
Coronary disease	1.5%	1.1%	1.3%	1.8%	0.772
Stroke	1.5%	1.1%	0.4%	0.7%	0.109
Osteoarthritis/arthritis	51.1%	48.4%	44.5%	44.4%	0.022
Cancer	1.8%	1.8%	2.2%	1.8%	0.875
Depression requiring treatment	4.8%	7.7%	7.9%	9.2%	0.015
Number of medications, mean ± SD	1.9 ± 1.9	1.9 ± 1.8	2.1 ± 2.1	1.9 ± 1.9	0.597

Notes: SD = standard deviation.

Quartiles ranges are: in men (0% to 9.55% for Q1; 9.56% to 15.80% for Q2; 15.81% to 24.28% for Q3; and 24.29% to 57.78% for Q4); in women (0% to 12.01% for Q1; 12.02% to 9.24% for Q2; 19.25% to 27.84% for Q3; and 27.85% to 79.66% for Q4).

Table 6. Frailty risk according to ultra-processed food consumption quartiles in the Seniors-ENRICA cohort study expressed as a percentage of total energy (% energy) or as gram per day/kg of body weight of each subject (g/kg). *N*=1,822.

	Ultra-processed food consumption (% energy)				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*p* Value for trend
N	456	455	456	455	
Frailty	17	24	43	48	
Model 1, OR (95% CI)	1 (Ref.)	1.51 (0.79-2.89)	2.90 (1.60-5.26)	3.22 (1.79-5.79)	<0.001
Model 2, OR (95% CI)	1 (Ref.)	1.53 (0.80-2.95)	2.92 (1.61-5.33)	3.52 (1.94-6.38)	<0.001
Model 3, OR (95% CI)	1 (Ref.)	1.52 (0.78-2.96)	2.98 (1.62-5.50)	3.67 (2.00-6.73)	<0.001
	Ultra-processed food consumption (g/kg)				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*p* Value for trend
N	456	455	456	455	
Frailty	20	36	39	37	
Model 1, OR (95% CI)	1 (Ref.)	2.23 (1.25-4.00)	2.21 (1.24-3.94)	2.17 (1.22-3.89)	0.017
Model 2, OR (95% CI)	1 (Ref.)	2.36 (1.31-4.24)	2.32 (1.30-4.14)	2.38 (1.32-4.28)	0.008
Model 3, OR (95% CI)	1 (Ref.)	2.42 (1.32-4.41)	2.48 (1.37-4.49)	2.57 (1.41-4.70)	0.004

Notes: OR = odds ratio; CI = confidence interval. The *p* values for the OR were obtained using logistic regression.

Model 1: Logistic regression model adjusted for sex and age (60–69, 70–79, 80 years or older).

Model 2: As in Model 1 and additionally adjusted for level of education (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (current, former, never smoker), and ex-drinker status (yes, no).

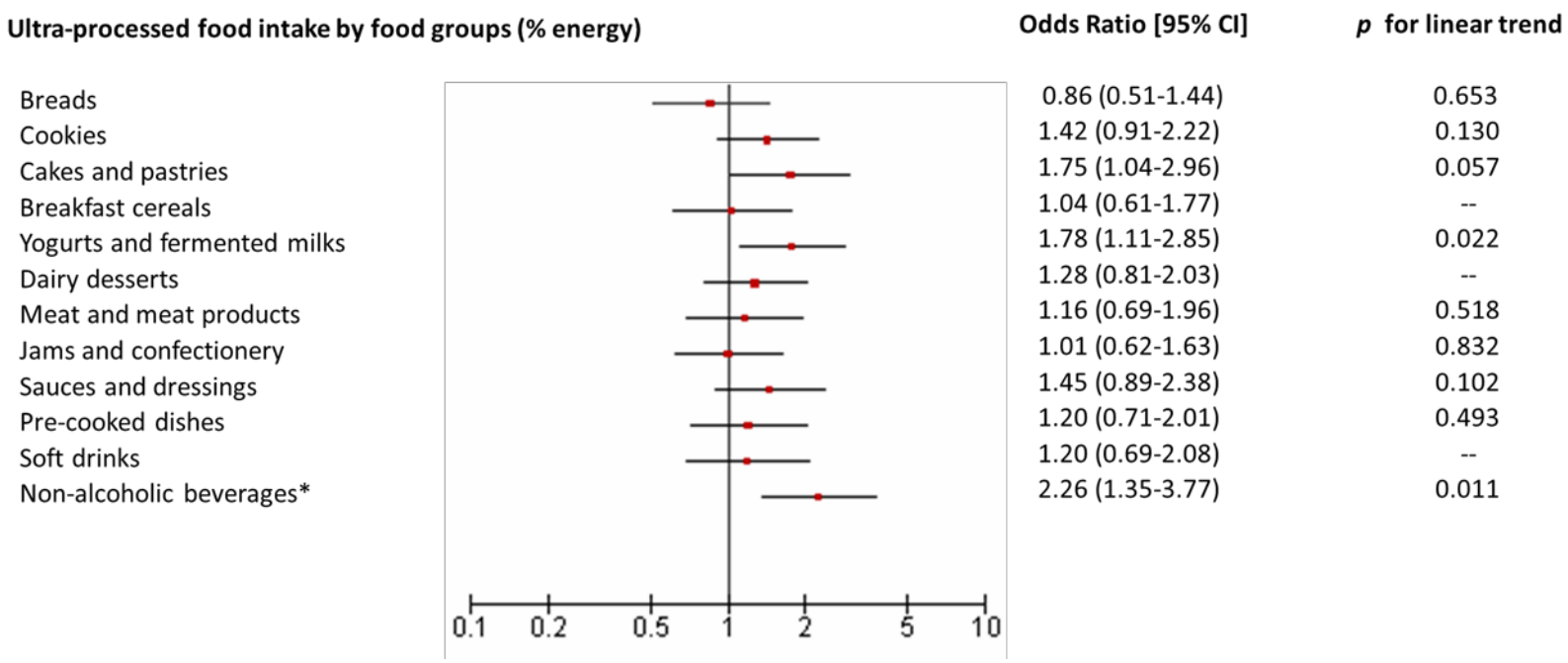
Model 3: As in Model 2 and additionally adjusted for chronic respiratory disease (yes, no), coronary disease (yes, no), stroke (yes, no), osteoarthritis/arthritis (yes, no), cancer (yes, no), depression requiring treatment (yes, no), and number of medications (continuous).

Table 7. Odds ratios of frailty components according to ultra-processed food consumption quartiles in the Seniors-ENRICA cohort study expressed as a percentage of total energy (% energy) or as gram per day/kg of body weight of each subject (g/kg). *N*=1,822.

	Ultra-processed food consumption (% energy)				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*p* Value for linear trend
Low physical activity, OR (95% CI)	1 (Ref.)	1.09 (0.75-1.60)	1.35 (0.94-1.96)	1.35 (0.93-1.96)	0.066
Slowness, OR (95% CI)	1 (Ref.)	0.82 (0.53-1.24)	1.64 (1.13-2.39)	1.74 (1.19-2.54)	<0.001
Unintentional weight loss, OR (95% CI)	1 (Ref.)	1.77 (0.94-3.32)	3.15 (1.75-5.66)	3.33 (1.86-5.97)	<0.001
Muscle weakness, OR (95% CI)	1 (Ref.)	1.05 (0.78-1.43)	1.11 (0.82-1.51)	1.06 (0.78-1.44)	0.661
Exhaustion, OR (95% CI)	1 (Ref.)	1.12 (0.74-1.70)	1.15 (0.76-1.75)	1.21 (0.80-1.83)	0.379
	Ultra-processed food consumption (g/kg)				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	*p* Value for linear trend
Low physical activity, OR (95% CI)	1 (Ref.)	1.36 (0.93-2.00)	1.41 (0.96-2.07)	1.87 (1.28-2.74)	0.002
Slowness, OR (95% CI)	1 (Ref.)	1.07 (0.71-1.63)	1.79 (1.21-2.63)	2.02 (1.37-2.97)	<0.001
Unintentional weight loss, OR (95% CI)	1 (Ref.)	1.66 (0.93-2.97)	2.28 (1.31-3.94)	2.46 (1.42-4.26)	<0.001
Muscle weakness, OR (95% CI)	1 (Ref.)	0.82 (0.61-1.12)	1.05 (0.78-1.42)	1.06 (0.78-1.44)	0.426
Exhaustion, OR (95% CI)	1 (Ref.)	1.26 (0.85-1.88)	0.93 (0.61-1.40)	1.03 (0.68-1.56)	0.734

Notes: OR = odds ratio; CI = confidence interval. Analyses adjusted as in Model 3.

Figure 2. Odds ratios (95% confidence interval) for quartile 4 (highest intake) of groups of ultra-processed food consumption as a percentage of total energy (% energy) or as caloric density (g/1000 kcal or mg/1000 kcal) versus quartile 1 (lowest intake), in the Seniors-ENRICA cohort study (*N*=1,822).



Notes: OR = odds ratio; CI = confidence interval.

When the intake of ultra-processed foods from a specific food group occurred in less than 50% of the participants, the ORs (95% CI) was calculated among tertiles (as in breads, cookies, cakes and pastries, yogurts and fermented milks, jams and confectionary, and non-alcoholic beverages). When the intake of ultra-processed foods from a specific food group occurred in less than 25% of the participants, the ORs (95% CI) were calculated between participants who consumed the food compared with those who did not (as in breakfast cereals, dairy desserts, and soft drinks). *P* for linear trend was calculating using quartiles as a continuous variable. Model adjusted for sex, age, level of education, marital status, tobacco consumption, ex-drinker status, chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, depression requiring treatment, and number of medications.

*Non-alcoholic beverages group includes instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks (Supplementary Table 2).

Supplementary Table 7. Total food items considered in the Seniors-ENRICA cohort according to NOVA classification.

1) Unprocessed or minimally processed foods

Cereals
- *Grains and flours*: Boiled white rice, boiled whole grain rice, corn flour, gofio (flour from roasted grains), rye flour, wheat flour, whole grain wheat flour, quinoa, wheat semolina, boiled couscous, corn flour.
- *Pasta*: boiled pasta (macaroni, spaghetti).
- *Breakfast cereals*: oat bran, wheat bran.

Milk and dairy products
- *Milk*: whole milk, whole goat's milk, sheep's milk, skimmed milk (0-1%), powdered skimmed milk, whole dry milk, low fat powdered milk (2%), whole milk (4%), whole fresh milk, semi skimmed milk (2%).
- *Yogurts and fermented milks*: natural skimmed fermented milk, natural fermented whole milk, natural skimmed yogurt, natural whole yogurt, kefir/ kephir milk.

Meat and meat products
- *Pork*: pork (ground meat with lard/bacon fat), lean pork, pork chops with fat, lean pork chops, pork ribs, pork fillets, pork sirloin, pork trotters.
- *Veal*: veal, lean veal, veal chops, veal ribs, veal rack, lean veal rump steaks (entrecote), veal shoulder blade steaks, veal round steaks, veal sirloin steaks.
- *Lamb*: lamb, lean lamb, lamb chops, lambs head, lean shoulder blade of lamb, leg of lamb.
- *Beef:* beef, beef rib-eye (entrecote), beef skirt steaks, boneless beef eye, beef sirloin steaks.
- *Poultry*: chicken, whole snipe, hens, skinless hens, skinless goose, whole duck, skinless duck, turkey (legs/drumsticks), skinless turkey legs/drumsticks, turkey breasts, skinless turkey, skinless pigeon, whole chicken, chicken drumsticks, skinless chicken drumsticks, whole chicken breasts, skinless chicken breasts, skinless chicken, partridge.
- *Offal*: offal, tripe, cows heart, lamb's liver, chicken liver, ox liver, veal's tongue, calf's sweetbreads, pig's kidneys, lamb's kidneys, calf's kidneys, pig's brain, lamb's sweetbreads.
- *Other meats and meat products*: horse meat, kid (goat), rabbit, wild pig (boar).

Eggs and egg products
Raw hen egg whites, hens egg, lightly pouched hen's eggs, fried hen's eggs, hard-boiled eggs, pouched hen's eggs, raw hen egg yolks, snipe's eggs.

Fish and fish products
- *White fish*: fillets of white fish, white fish, haddock, blue whiting, cod, red bream, scorpion fish, dogfish/shark marinated in vinegar and oil, black sea bream, ribbon fish, gilt-head bream, bay whiff, halibut, sole, seabass, whiting, hake, frozen hake, grouper fish/sea perch, grey mullet, Breca fish/Pandora fish, small whiting/young hake, small frozen whiting, catfish, flounder, monkfish, skate/ray, turbot, red mullet, white seabream, shark.
- *Blue fish*: blue fish, eel, tuna, albacore/longfin tuna, bonito (tuna), anchovy, mackerel,

whitebait, conger eel, emperor fish, pompano fish, blue whiting, red bream/red porgy, small fried whitebait, swordfish, salmon, sardines, baby eels.
- *Freshwater fish*: pike, perch, trout.
- *Other fish and fish products*: caviar from sturgeon/roe, lumpfish roe, dried salted tuna.

Seafood
- *Crustaceans*: lobsters, crabs, freshwater crayfish, spider crabs, langoustine/Dublin bay prawns, prawns/shrimp, frozen prawns/shrimp, baby shrimp, rock lobsters, jumbo shrimp, small crabs, barnacles/goose barnacles.
- *Molluscs*: clams, cockles, winkles, squid, sea snails, baby squid, baby clams, mussels, razor shells/razor clams, oysters, octopus, cuttlefish, scallops.

Vegetables
- *Steam and leaf vegetables:* chard/Swiss chard, celery, watercress, cabbage, borage (starflower), cardoon, chives, white cabbage, Brussels sprouts, red cabbage, green cabbage, endives, curly endives, raw asparagus, boiled green asparagus, cooked spinach, raw spinach, green turnips, fennel, lettuce.
- *Root vegetables*: parsnips, radishes, beetroot, soya sprouts/soya shoots, carrots.
- *Bulbs vegetables*: garlic, green garlic/garlic shoots, onions, leaks,
- *Inflorescence fruit and vegetables*: artichokes, aubergines, broccoli, courgettes, pumpkin, cauliflower, frozen cauliflower, frozen green beans, boiled green beans, corn, cucumbers, peppers, red peppers, green peppers, tomatoes.
- *Mushrooms*: mushrooms, milk cap mushrooms/red pine mushrooms, brewer's mushrooms/wild fungus/edible fungus.

Legumes and legume products
- *Fresh legumes*: frozen boiled peas, boiled peas, broad beans.
- *Pulses*: boiled white beans, boiled black beans/frijoles, boiled chickpeas, beans, boiled lentils, boiled soya, boiled beans, boiled canned beans.
- *Legume's derivate products*: soya flour, soya milk.

Tubers and tuber products
Potatoes, roasted potatoes, boiled potatoes, sweet potatoes, tapioca, yams.

Fruit and fruit products
- *Fresh fruit*: apricots, cranberry, kaki, cherries, apple custard, whole prunes, raspberries, strawberries, pomegranate, gooseberries, prickly pears, figs, kiwis, litchi, limes, lemons, fresh lemon juice, mandarines/tangerines, mango, whole roast apples, whole apples, peeled golden delicious apples, peeled apples, peeled starking apples, fruit, peaches, melons, quince, blackberries, oranges, fresh orange juice, nectarines, peeled medlar fruit, papayas, Paraguayan, whole pears, peeled pears, pineapples, bananas, grapefruit, fresh grapefruit juice, watermelon, black grapes, green grapes.
- *Dried fruit*: dried apricots, dried plums, dried dates, dried figs, dried peaches, raisins.
- *Oleaginous fruit*: avocado.

Nuts
Nuts, raw almonds, toasted almonds, hazelnuts, chestnuts, toasted chestnuts, raw tigernuts, fresh coconuts, dried coconuts, nuts, peanuts, unsalted sunflower seeds, sesame seeds, cashew nuts, macadamia nuts.

Non-alcoholic beverages
- *Water*: bottled sparkling water, water/tab water, mineral water, still water,
- *Coffee and brews*: decaffeinated coffee made with an Italian coffee maker, decaffeinated expresso, filtered decaffeinated coffee, expresso made with an Italian coffee maker, expresso, filtered coffee, tea/herbal tea, tea with milk, tea, wheat bran.

Miscellanea
Fresh yeast.

2) Processed culinary ingredients

Oils and fats
- *Oils:* oil, seed oil, canola oil, sunflower oil, sunflower and olive oil 50%, corn oil, olive oil, olive oil (extra virgin), soya oil.
- *Fats*: lard, butter.

Sugars and sugary products
- *Sugars*: white sugar, brown sugar, fructose, honey.

Condiments
Cinnamon, chili peppers/chili peppers, fresh parsley, paprika, ground pepper, table salt, vinegar.

3) Processed foods

Cereals
- *Breads:* white bread, biscotte/rusk bread/breadsticks, french stick, french stick without salt, white sliced bread, french stick/baguette, loaf of bread/ciabatta, mixed wheat and rye bread, whole grain bread.

Milk and dairy products
- *Fresh cheeses*: coat's cheese, fat free fromage frais, mixed goat's and cows's cheese, fresh cheese in tubs, mozzarella cheese (buffalo milk), fresh coat's cheese, cottage cheese.
- *Cured cheeses*: semi-cured cheese, soft cheese, blue cheese, babybel cheese/edam cheese, brie cheese, camembert 20-30% fdm (fat content in dry matter), camembert 40-50% fdm (fat content in dry matter), camembert 60% fdm (fat content in dry matter), aged goat's cheese, cheddar cheese, soft cow's cheese, edam cheese, goat's cheese with mould, cured goat's cheese, feta cheese (ewe and goat's milk), semi-cured goat's cheese, blue cheese, sheep and goat's cheese, sheep, cow and goat's cheese, sheep and cow's cheese, edam cheese, emmenthal cheese, gouda cheese, gruyere cheese, unpasteurized goat's cheese, soft to hard white cow's milk cheese, goat's cheese (milky and nutty flavour), manchego cheese/sheep's cheese, cured manchego cheese/cured sheep's cheese, sheep's cheese in oil, semi-cured sheep's cheese, muenster cheese, cream cheese, parmesan cheese, goat's cheese, sheep's cheese, roquefort cheese, cow's cheese, tetilla cheese (cow's milk), torta del casar cheese (sheep's milk), zamorano cheese (hard sheep's cheese), daised semi-cured goat's cheese, daised cured goat's cheese, mould daised ripened semi-cured goat's cheese, diced fresh cheese, diced semi-

cured cheese, diced cured cheese.

Meat and meat products
- *Cold meats*: corned beef/dried beef, ham cured in salt with fat/tocino, ham cured in salt without fat/tocino, dried pork sirloin.
-*Other* m*eat products*: bacon, smoked bacon, homemade hamburgers, homemade grounded/minced meat, stewed hare with sauce, fried blood with onions.

Eggs and egg products
Scramble eggs, French omelette.

Fish and fish products
Smoke fish, salted fish, salted cod, small fried whiting, anchovies in oil, kippers, canned albacore in oil, canned natural tuna, canned tuna in oil, canned tuna marinated in vinegar, canned natural bonito (tuna), canned bonito in oil, canned bonito cooked and marinated in vinegar, anchovies in vinegar, canned mackerel in oil, smoked salmon, canned sardines in oil, canned marinade sardines, smoked trout.

Seafood
Canned natural crabs, natural canned cockles, canned marinated mussels.

Vegetables
Canned celery, canned white asparagus, palm hearts, canned soya sprouts, canned carrots, canned beetroot, canned green beans, canned boiled corn, canned tomatoes, canned artichokes, red bell peppers, canned mushrooms, mixed canned vegetables/diced vegetables.

Legumes and legume products
Canned peas, canned white beans, canned chickpeas, canned lentils, tofu.

Tubers and tuber products
Homemade fried potatoes.

Fruit and fruit products
- *Fruit preserves*: fruit in syrup, guava in syrup, fruit cocktail in syrup, peaches in syrup, pears in syrup, pineapples in syrup, pineapples in natural juice.
- *Oleaginous fruit*: olives, black olives, green olives.

Nuts
Fried salted almonds, fried salted peanuts, nuts and raisins, salted toasted pistachio nuts, salted toasted peanuts, roux.

Sauces and dressings
Natural fried tomatoes sauce.

Homemade cooked food
Homemade lasagna, homemade lasagna with bechamel, sautéed peppers, onions and tomatoes, fish, onion soup, tapioca soup/tapioca dips.

Snacks

Capers, lupines, large capers, canned baby or pearl onions in vinegar, pork crackling snacks, mixed pickles, toasted chickpeas, canned pickles in vinegar/ pickled gherkins.

Non-alcoholic beverages
Almond milk, natural tomato juice.

Alcoholic beverages
Beer, stout beer, lager, cider, sweet cider, dry cider, wine, sparkling wine/champagne, sparkling very dry white wine, white wine, sherry, rosé wine, red wine, brewer's yeast/beer yeast.

4) Ultra-processed food and drink products

Cereals:
- *Pasta*: boiled pasta with eggs, meet ravioli, cheese ravioli.
- *Breads:* crispbread, whole grain crispbread, white sliced bread, hamburger buns, hot dog buns, Vienna style loaf of bread, brown sliced bread, bread crumbs, pizza bases, croutons.
- *Cookies*: biscuits, chocolate chip cookies, chocolate filled biscuits, tea biscuits, whole grain crackers, tea biscuits.
- *Cakes and pastries*: tea cakes, pastries, whole grain pastries, cake, lady fingers/sponge fingers/sponge cake, tea buns/soft bread buns, chocolate filled buns, custard filled buns, cream buns, Swiss buns, choux buns, chocolate Danish pastry, churros, dried fruit sponge cake, croissant, chocolate filled croissants, cheese filled croissants, doughnuts, Mallorcan Danish pastry, fairy cakes/cupcakes, short crust pastry, puff pastry, lard, meringue, puff pastry filled with crème and custard, Danish pastry, puff pastry, Danish pastry filled with jam, chocolate cake, sponge cake, cream cake, pastry filled with custard, plum cake, homemade doughnuts, cake filled with cream, fruit cake, whisky cake, apple pie, cheese cake, egg pudding, dried fruit sponge cake, sponge cake, chocolate sponge cake, tea buns, butter sponge cake, Madeira cake, choux pastry with custard, fruit Danish pastry, raisin pudding, apple pie with custard.
- *Breakfast cereals*: cereals, puffed rice/rice krispies, bran flakes/all-bran, chocolate cereal flakes, crunchy/honey cereal flakes, corn flakes, muesli, puffed wheat.

Milk and dairy products
- *Milkshakes*: chocolate milk, milkshakes, sweet condensed milk, evaporated milk/carnation milk, cooking cream.
- *Yogurts and fermented milks*: skimmed fermented milk with fruit, whole fermented milk with fruit, skimmed flavoured yogurt, skimmed yogurt with fruit, natural skimmed yogurt with sweetener, whole flavoured yogurt, whole yogurt with fruit, natural whole yogurt with sweetener, whole drinking/liquid yogurt, whole drinking/liquid yogurt with fruit, natural drinking/liquid yogurt with sweetener, natural Greek yogurt.
- *Dairy desserts*: custard, pastry cream, curd, egg flan with crème caramel, vanilla flan, chocolate ice cream, creamed ice cream, vanilla ice cream, ice cream, flavoured commercial mousse, custard, cream brulée, chocolate mousse, chocolate custard, natural petit Suisse with sweetener.
- *Ultra-processed cheeses:* flavoured petit Suisse, babybel cheese/edam cheese, raclette cheese (semi-hard cow's milk), sliced cheese, processed cheese in portions, processed light cheese, cheese spread/cheese dip, extra creamy melting cheese, creamy melting

cheese, cheese spread, soft cheese type Philadelphia/cheese spreads, grated parmesan cheese, light cheese.
- *Creams:* whipping cream, whipping cream with sugar.

Meat and meat products
- *Meats:* ground/minced meat, pork (ground/minced meat), ground/minced veal.
- *Cold meats:* chistorra, chorizo/dried sausages, fresh chorizo/fresh sausages, chorizo, salami, dried sausages, pork sausages, chorizo, sausage, chorizo spread, cold meats, bratwurst, Catalan sausages, wild pig (boar) jellied sausage, cold meat galantine, foie-grass, boiled ham, shoulder of ham, blood pudding/black pudding, blood pudding/black pudding, cold sausage type salami, smoked pancetta, pigs liver-pate, turkey slices, salami, Frankfurt sausages, Catalan sausages, blood pudding with rice, champagne pate/country pate.
- *Other meat products*: pork meatballs, meatballs, seasoned ground/minced meat for stuffing, chicken hamburgers, beef hamburgers, restaurant/industrial hamburgers, chicken sausages, fresh sausages, canned meatballs.

Fish and fish products
Surimi/crab sticks, surimi.

Oils and fats
- *Fats:* margarine (75% from vegetables), light margarine, mixed margarine (50% from animals, 50% from vegetables), vegetable margarine.

Legumes and legume products
Soya milkshake.

Tubers and tuber products
Potato puree made with milk, potato puree made with water.

Fruit and fruit products
Apple compote with sugar/stewed apples with sugar.

Nuts
Peanut butter.

Sugars and sugary products
- *Jams and confectionery:* jam, low calorie jam, sweets/candy, chewing gum, sugary almonds, ice pops, liquor ice, sorbet, chocolate, chocolate wafer biscuits, chocolate caramel bars, chocolate bars, chocolates/bonbons/bon-bon, drinking chocolate/cocoa, white chocolate, milk chocolate, milk chocolate with nuts, dark chocolate, dark chocolate with nuts, cooking chocolate, chocolate hazelnut spread, chocolate truffles, nougat, marzipan, hard nougat, soft nougat, apple pudding/apple sauce, sweet quince jelly/quince jelly, caramel coated almonds.

Sauces and dressings
Garlic mayonnaise, ketchup, olive oil mayonnaise, sunflower oil mayonnaise, soya oil mayonnaise, mayonnaise, light mayonnaise, mustard, blue cheese sauce/Roquefort sauce, barbeque sauce, béarnaise sauce, béchamel sauce, Bolognese sauce, carbonara sauce, almond sauce, soya sauce, hollandaise sauce, mornay sauce, gravy, cocktail

sauce, tartar sauce, green sauce, parsley sauce, industrial fried tomato sauce, sweet and sour sauce.

Pre-cooked dishes
Frozen fried floured squid, fried pastries baked meats (frozen), fried pastries, frozen baked cheese, frozen bread crumbed fish, fried spring rolls (frozen), frozen croquettes, frozen bread crumbed chicken, fried bread crumbed ham with cheese, frozen meat cannelloni with béchamel, frozen lasagna, frozen tomato and cheese pizza, canned squid in spicy tomato sauce, canned meat ravioli with tomato sauce, canned leek soup, canned bean stew with Spanish sauce, canned gazpacho (cold Spanish tomato soup), hotdogs with mustard, cheese hamburgers with buns, hot ham and cheese sandwiches, croquettes, meats in pastry, cod fritters, meat and vegetable stock/soup, fish stock/soup, vegetable stock/soup, homemade cannelloni with béchamel, tomato soup, ham (chicken or meat croquettes), fish croquettes, tuna pastries, meat pastries, chicken soup with noodles, packed soup.

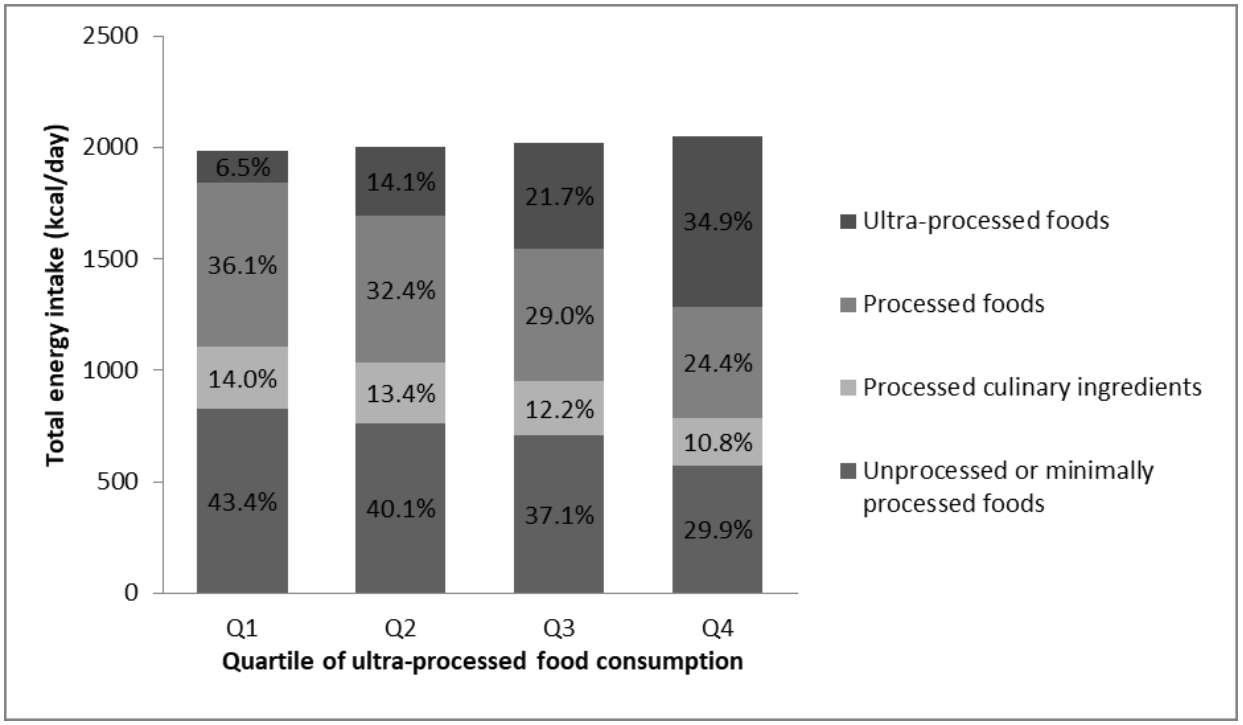
Salty snacks
Packaged snacks, corn snacks, salted crackers, salted cheese crackers, crackers, salted popcorn (with oil), popcorn (without oil), potato crisps/chips, low-fat potato crisps/chips, flavoured corn snacks, wotsits, Cheetos.

Non-alcoholic beverages
- *Instant coffee and cocoa*: cocoa/powdered drinking chocolate, instant decaffeinated coffee, instant coffee, eko/instant malt coffee substitute without water.
- *Soft drinks*: beverage/soft drinks/sodas, lemonade, sparkling lemon and lime flavoured soft drinks, sparkling cola soft drinks, sparkling diet cola soft drinks, sparkling lemon flavoured soft drinks, still lemon flavoured soft drinks, sparkling orange flavoured soft drinks, still orange flavoured soft drinks, tonic water, lemon flavoured slush puppy.
- *Packaged juices:* apricot juice/apricot nectar juice, exotic fruit juice/exotic fruit nectar juice, packaged mango nectar juice, packaged passion fruit nectar juice, packaged orange nectar juice, packaged pear nectar juice, packaged exotic fruit juice, packaged lime juice, packaged apple juice, packaged orange juice, packaged pear juice, packaged pineapple juice, packaged grapefruit juice, packaged tomato juice, packaged grape juice, packaged carrot juice.
- *Other non-alcoholic drinks*: isotonic drinks, bitter soda soft drinks without alcohol, alcohol free beer, powdered almond milk, tiger nut milk, grape juice, fruit juice, energy drinks.

Alcoholic drinks
Bitter soda with alcohol, sangria, vermouth, sweet black vermouth, sweet wine/dessert wine, alcoholic liqueur, alcoholic fruit liqueur, clear fruit liqueur, dark fruit liqueur, sloe gin/sloe brandy, schnapps, dry anise, brandy/cognac, baileys/Irish cream, gin, rum, tequila, vodka, whisky/whiskey.

Miscellanea
Sweetener, jelly.

Supplementary Figure 1. Total energy intake (kcal/day) according to quartiles of ultra-processed food consumption and relative contribution of each NOVA category of food processing (as a percentage of total energy) to total dietary intake (*N*=1,822).



5.4. DISCUSSION

After 3.5 years of follow-up, ultra-processed food intake was associated with an increased risk of frailty in a cohort of a noninstitutionalized population aged 60 or older from Spain. Participants in the highest quartile of total energy intake from ultra-processed foods have a tripled risk of incident frailty than participants in the lowest quartile of consumption.

A recent systematic review confirmed the importance of both quantitative (e.g. amount of energy intake) and qualitative factors (e.g. nutrient quality) in the development of the frailty syndrome in the elderly (148). Ultra-processed foods are palatable, ready to eat, and with a very high energy density (66-69). These characteristics make ultra-processed foods more likely to be over-consumed, which may consequently increase total dietary energy intake among consumers. Likewise, ultra-processed food consumption has been associated with a poorer diet quality (less fiber, fruit, and vegetable intake), that also contributes to frailty (149, 150). Results are in line with previous analyses of this cohort, in which a high adherence to the Mediterranean diet (low in ultra-processed foods) was inversely related to incident frailty (80). Also, a Prudent dietary pattern showed an inverse dose-response association with frailty incidence, while a Westernized dietary pattern had a positive association with some of their components (such as high consumption of dairy products, as well as red and processed meat) (141). In both cases, the intake of dairy products and red meat was detrimental.

Our findings concur with those obtained in previous research by Laclaustra et al (145). Association with frailty was strongest for sugars added during food production, while the intake of sugars naturally appearing in foods was not associated with frailty. The amounts of added sugars from pastries and cookies (15% of the total added sugars) and sugary or sweetened beverages (6% of the total added sugars) were positively associated with the risk of frailty. Also, the frailty criterion of unintentional weight loss increased dose dependently with added sugars.

Interestingly, yogurts and fermented milks is, after non-alcoholic beverages, the food group from ultra-processed foods more positively related to frailty and has

important content of animal protein, simple sugars, as well as SFAs (82).

Regarding yogurts and fermented milks, we found mixed results among studies. It has been previously reported with data from this cohort that the consumption of yogurt and whole milk was linked to a greater risk of frailty. Similar results were obtained when these foods were analyzed separately. However, a higher consumption of low-fat yogurt and low-fat milk was not linked to a lower incident frailty (143). These results are not totally comparable with our findings, where yogurts and fermented milks included as ultra-processed were those sugary, sweetened, flavored milks, independent of their amount of fat content. On the other hand, among 106,772 individuals from Sweden, the intake of fermented milk products (such as yogurt, sour milk, and cheese), was associated with lower rates of fracture and mortality in women. Risk reductions in men were modest or non-existent (151). In the aforementioned study, the endpoint was fracture rates (an outcome highly dependent on calcium intake), not frailty, and the participants were younger than in our cohort. Moreover, the difference between ultra-processed and non ultra-processed dairy products was not made. In the future, a comprehensive assessment of the association between dairy products and health among the elderly is needed. So far today, this relationship is controversial. We suggest new studies considering both, the fat content of dairy products and their processing level, before making dietary recommendations.

The study's strengths include its prospective design and the estimation of dietary intakes through a validated dietary history. Trained staff collected a wide range of food items. Other advantages were the adjustment for a large number of potential confounders and the robustness of the results that did not change substantially after running several sensitivity analyses. Some limitations should be noted. First, as in most nutritional epidemiology studies, a certain recall bias cannot be ruled out because diet was self-reported. Second, although there was agreement among authors about NOVA classification of our participants'diet, certain ultra-processed food misclassification cannot be ruled out. Third, the number of frailty cases was relatively small, but we still found quite strong associations. Fourth, the low number of consumers in some ultra-processed food groups (such as breakfast cereals, dairy desserts, and soft drinks) may not allow to achieve statistical significance in those groups. Finally, although we adjusted for the potential confounders, some residual confounding may persist.

In conclusion, in this prospective study of community-dwelling older adults from Spain, a greater intake of ultra-processed foods from non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks), yogurts and fermented milks (natural and non-sugary yogurts are not included in this category), as well as cakes and pastries was significantly associated with incident frailty. This association was strong and independent of sociodemographic, lifestyle, and morbidity factors. These findings add evidence for the restriction of ultra-processed foods, and the promotion of fresh or minimally processed foods in the older adult population.

6. CONCLUSIONS

Conclusion 1:

Total protein, animal protein, as well as MUFA intake was inversely associated with incident frailty in community-dwelling older adults aged 60 years or older. However, no association was found between the intake of vegetable protein, saturated fats, long-chain ω-3 fatty acids, α-linolenic acid, linoleic acid, simple sugars, or polysaccharides and the risk of frailty. Our findings also support that the recent recommendation of protein intake averaging 1.20/g/kg/d might prevent frailty.

Conclusion 2:

A higher total protein intake was associated with lower risk of falls in community-dwelling older adults who had experienced an unintentional weight loss of 4.5 kg or more in the preceding year.

Conclusion 3:

A higher intake of ultra-processed foods was associated with an increased risk of frailty in community-dwelling older adults. This association was specifically observed for non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks), yogurts and fermented milks (natural and non-sugary yogurts are not included in this category), as well as cakes and pastries.

CONCLUSIONES

Conclusión 1:

El consumo de proteínas animales, proteínas totales así como de ácidos grasos monoinsaturados estaba inversamente asociado a fragilidad incidente en individuos no institucionalizados de 60 o más años. Sin embargo, no se encontró asociación entre el consumo de proteínas vegetales, ácidos grasos saturados, ácidos grasos ω-3 de cadena larga, ácido α-linolénico, azúcares simples así como azúcares polisacáridos y el riesgo de fragilidad. Nuestros hallazgos también apoyan las recomendaciones recientes de que un consumo medio de proteínas de 1.20/g/kg/día podría prevenir la fragilidad.

Conclusión 2:

Un mayor consumo de proteínas totales estaba asociado a un menor riesgo de caídas en adultos mayores no institucionalizados que habían experimentado una pérdida de peso no intencional de 4.5 Kg o más en el año precedente.

Conclusión 3:

Un mayor consumo de alimentos ultra-procesados estaba asociado a un riesgo aumentado de fragilidad en adultos mayores no institucionalizados. Esta asociación se observó específicamente para bebidas no alcohólicas (café y cacao instantáneo, zumos envasados y otras bebidas no alcohólicas excluyendo los refrescos), los yogures y las leches fermentadas (los yogures naturales y los no azucarados no están incluidos en esta categoría), así como las tartas y la pastelería.

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Research Article

Macronutrients Intake and Incident Frailty in Older Adults: A Prospective Cohort Study

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Abstract

Background: Only a few studies have assessed the association between protein intake and frailty incidence and have obtained inconsistent results. This study examined the association of protein and other macronutrient intake with the risk of frailty in older adults.
Methods: A prospective cohort of 1,822 community-dwelling individuals aged 60 and older was recruited in 2008–2010 and followed-up through 2012. At baseline, food consumption was assessed with a validated, computerized face-to-face diet history. In 2012, individuals were contacted again to ascertain incident frailty, defined as the presence of at least three of the five Fried criteria: low physical activity, slowness, unintentional weight loss, muscle weakness, and exhaustion. Analyses were performed using logistic regression and adjusted for the main confounders, including total energy intake.
Results: During a mean follow-up of 3.5 years, 132 persons with incident frailty were identified. The odds ratios (95% confidence interval) of frailty across increasing quartiles of total protein were 1.00, 0.55 (0.32–0.93), 0.45 (0.26–0.78), and 0.41 (0.23–0.72); p trend: .001. The corresponding figures for animal protein intake were 1.00, 0.68 (0.40–1.17), 0.56 (0.32–0.97), and 0.48 (0.26–0.87), p trend: .011. And for intake of monounsaturated fatty acids (MUFAs), the results were 1.00, 0.66 (0.37–1.20), 0.54 (0.28–1.02), and 0.50 (0.26–0.96); p trend: .038. No association was found between intake of vegetable protein, saturated fats, long-chain ω-3 fatty acids, α-linolenic acid, linoleic acid, simple sugars, or polysaccharides and the risk of frailty.
Conclusion: Intake of total protein, animal protein, and MUFAs was inversely associated with incident frailty. Promoting the intake of these nutrients might reduce frailty.

Key Words: Frailty, Energy intake, Macronutrients, Protein, Monounsaturated fatty acids

Frailty is a medical syndrome characterized by sarcopenia, decreased functional reserve, and augmented vulnerability to even minor stressors (1). As a result, frail individuals are at increased risk of disability, institutionalization, and death (2). Due to the progressive ageing of the population in industrialized countries, prevention strategies should focus on delaying frailty, reversing the frailty process, and reducing the adverse health outcomes of the frailty syndrome (3).

Poor nutrition plays a key role in the frailty process (4). However, research on the relationship between macronutrient intake and incident frailty is scarce. To our knowledge, only two cohort studies have assessed this association. The first one is the Women's Health Initiative Observational Study (WHI-OS) (5), conducted with 24,417 women aged 65 or older. In this study, a 20% higher protein intake was associated with a 32% lower risk of frailty in the subsequent 3 years. This association was independent of the source (animal or vegetable) and quality (essential amino acid intake) of protein. However, no information on other macronutrients was reported. The second study is the Osteoporotic Fractures in Men (MrOS) study (6), where no association was found between carbohydrate, fat, or protein intake and risk of frailty among 2,423 men aged 65 or older followed during 4.6 years. However, the statistical power of this analysis was limited because only 25 frail men were identified.

Therefore, this study examined the association of protein and other macronutrient intake with the risk of frailty in noninstitutionalized older men and women from Spain.

Methods

Study Design and Population

Data were taken from the Seniors-ENRICA (Study on Nutrition and Cardiovascular risk factors in Spain) cohort, which was established in 2008–2010 with 2,614 community-dwelling individuals aged 60 years and older in Spain (7,8). At baseline, a computer-assisted phone interview was performed to collect data on sociodemographic factors, health behaviors, and morbidity. Then, two home visits were conducted to obtain a diet history, perform a physical exam, and collect biological samples; the average time between the phone interview and the second home visit was 2 weeks. For cognitively impaired participants, data were collected with the help of a proxy. Participants were followed-up until 2012, when a second wave of data collection was performed. Ninety-five participants (3.6%) died during follow-up; from the remaining 2,519 participants, 2,085 had complete information on frailty (7,8). Of them, we excluded 174 for being frail or having missing values for frailty at baseline, 8 with unreliable information on diet, and 81 who lacked data on diet or potential confounders. Thus, the analyses were performed with 1,822 participants. Compared with individuals included in the analyses, those not included were less often males, were slightly older, and had more frequent chronic conditions (Supplementary Table 1).

Participants gave written informed consent. The study was approved by The Clinical Research Ethics Committee of "La Paz" University Hospital in Madrid (Spain).

Study Variables

Diet

At baseline, habitual food consumption was collected through a validated computerized face-to-face diet history (9), which was developed from that used in the EPIC-Spain cohort study. Collected information included all foods consumed at least once every 15 days during the preceding year. The diet history registers 860 foods and 30 different cooking methods and uses more than 120 photographs to help in choosing the portion sizes. The intake of animal protein, vegetal protein, monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), long-chain ω-3 fatty acids, α-linolenic acid (ALA), linoleic acid (LA), simple sugars, and polysaccharides was estimated with standard food composition tables.

Frailty

Frailty was assessed according to the definition developed by Fried and colleagues (1). Specifically, frailty was defined as having at least three of the following five criteria: (i) low physical activity, defined as walking ≤2.5 hour/week in men and ≤2 hour/week in women; (ii) slowness, defined as the lowest quintile in our study cohort for the 3-m walking speed test, adjusted for sex and height; in men, the cutoff points were 0.47 and 0.45 m/second in those with height ≤173 and >173 cm, respectively; corresponding values in women were 0.37 and 0.40 m/second; (iii) weight loss, evaluated as involuntary loss of ≥4.5 kg in the preceding year; (iv) muscle weakness, defined as the lowest quintile of grip strength within groups defined by sex and body mass index (BMI) in our cohort. Strength was measured with a Jamar dynamometer, and we selected the highest value in two consecutive measurements; in men, the cutoff points were <29, <30, and <32 kg, respectively, in those with BMI ≤24, 24.1–28, and >28 kg/m^2; the corresponding values in women were <17, <17.3, <18, and < 21 kg, respectively, in those with BMI ≤23, 23.1–26, 26.1–29, and >29 kg/m^2; and (v) exhaustion, defined as responding "at least 3 to 4 days a week" to any of the following two questions taken from the Center for Epidemiologic Studies-Depression scale (10): "I felt that anything I did was a big effort" or "I felt that I could not keep on doing things."

Other Variables

At baseline, data on other sociodemographic factors, lifestyle, and morbidity were collected. Participants reported their sex, age, level of education, marital status, tobacco consumption, leisure-time physical activity (11), and physical activity in the household (11). Weight, height, and waist circumference were measured under standardized conditions. BMI was calculated as weight in kilograms divided by square height in meters. Abdominal obesity was defined as waist circumference greater than 102 cm in men and greater than 88 cm in women. Study participants also reported whether they suffered from any of the following physician-diagnosed prevalent diseases: chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, diabetes, and depression requiring treatment. Participants also reported the use of medications, which was checked against the drug packages. Finally, their ex-drinker status was self-reported.

Statistical Analysis

The associations between macronutrient intake and frailty were summarized with odds ratios (ORs) and their 95% confidence interval (CI), obtained from logistic regression. The dose–response relationship was tested with a p for linear trend, obtained by modeling quartiles of nutrient intake as a continuous variable. Analyses were energy adjusted with the nutrient residual model (12). Dietary variables were log transformed (13), and the residuals were obtained by regressing nutrient intake on total energy. The residuals of all sources of energy were then included in the model except for carbohydrates. Total energy intake was also included as a covariate. In this residual model, the coefficient for a certain nutrient is interpreted as the effect of substituting the amount of energy proceeding from such nutrient for the same amount of energy from carbohydrates, while holding constant the total energy intake and the intake of other macronutrients in the model (12). Conversely, to assess the association with carbohydrates, protein intake was excluded from the model. Macronutrients were modeled as sex-specific quartiles.

Three models were built with progressive adjustment for the main confounders. We selected the potential confounders a priori, because they were associated with either diet or frailty in the literature. The first model was adjusted for sex, age (60–69, 70–79, 80 years and older), total energy intake (kcal/day), the studied macronutrients, and ethanol intake (sex-specific quartiles). Model 2 was additionally adjusted for education (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (never, former, current smoker), BMI (<25, 25–29.9, ≥30 kg/m^2), abdominal obesity, and fiber intake (sex-specific quartiles). Finally, Model 3 was further adjusted for chronic morbidity: chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, diabetes, depression requiring treatment, and ex-drinker status.

Finally, among participants who were free of all frailty criteria at baseline, we assessed the association between macronutrient intake and each frailty criterion, using the same type of modeling.

Statistical significance was set at two-sided p less than .05. The analyses were conducted with Stata/SE, version 11.1 (StataCorp, College Station, TX).

Results

The mean age of participants was 68.7 years (*SD* = 6.4 years), and 48.7% were men. Over a mean follow-up of 3.5 years, 132 individuals with incident frailty were identified. Compared with non-frail individuals, those with frailty were less often males, were older, had a higher educational level, were more likely to smoke tobacco, and showed a higher frequency of obesity and other chronic conditions (Supplementary Table 2). Also, they had lower intake of energy and of most nutrients (Supplementary Table 3). Supplementary Table 4 shows the baseline characteristics of the study participants according to the studied macronutrients.

In fully adjusted analyses, the ORs (95% CI) of frailty across increasing quartiles of total protein were 1.00, 0.55 (0.32–0.93), 0.45 (0.26–0.78), and 0.41 (0.23–0.72); *p* trend: .001. As in most nutritional analyses, the explained variance was low. Specifically, the proportion of the variance of frailty risk explained by total protein was 1.30%. The ORs (95% CI) of frailty risk across quartiles of animal protein intake were 1.00, 0.68 (0.40–1.17), 0.56 (0.32–0.97), and 0.48 (0.26–0.87), *p* trend: .011. The proportion of the variance of frailty risk explained by animal protein intake was 0.79%. There was an inverse trend between vegetable protein intake and the risk of frailty, although the ORs did not reach statistical significance (Table 1).

When MUFAs were considered, the ORs (95% CI) for frailty according to increasing quartiles of intake were 1.00, 0.66 (0.37–1.20), 0.54 (0.28–1.02), and 0.50 (0.26–0.96); *p* trend: .038 (Table 2). The proportion of the variance of frailty risk explained by MUFA intake was 0.36%.

To assess the robustness of the results, we ran a number of sensitivity analyses. Results for protein and MUFA intake were similar when a ≥5% loss of body weight was used as frailty criterion, when we removed fiber intake from Model 2 to rule out overadjustment, when protein intake was expressed as g/kg/day, and when we excluded abdominal obesity from Model 2 to exclude the influence of muscle lipid accumulation on frailty. Finally, we did not find an interaction between macronutrient intake and age (<75 vs ≥75 years), based on likelihood ratio tests across quartiles of nutrient intake. The corresponding *p* values for interaction were .79 for total protein intake, .59 for animal protein intake, and .30 for MUFA intake.

No statistically significant association was found between incident frailty and intake of SFAs, long-chain ω-3 fatty acids, ALA, or LA (Table 2). Neither was it observed for total carbohydrates, simple sugars, or polysaccharides (data not shown). Results were similar in the sensitivity analyses (data not shown).

Regarding the frailty components, the OR (95% CI) of slow walking speed among those in the highest versus the lowest quartile of total protein intake was 0.63 (0.41–0.96); *p* for trend .022. The corresponding figure for animal protein intake was 0.45 (0.30–0.69); *p* for trend less than .001. Likewise, MUFA intake was inversely associated with unintentional weight loss; the OR (95% CI) was 0.45 (0.24–0.82) for the highest versus the lowest quartile; *p* for trend .012 (Supplementary Table 5). Results were also in the same direction for the rest of the frailty criteria, except for muscle weakness.

Table 1. ORs (95% CI) of Incident Frailty According to Quartiles of Protein Intake in the Seniors-ENRICA Cohort Study (*N* = 1,822)

	Frailty cases / *n*	Model 1	Model 2	Model 3
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Total protein				
Quartile 1 (lowest)	38/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	37/455	0.60 (0.37–0.98)*	0.62 (0.37–1.03)	0.55 (0.32–0.93)*
Quartile 3	31/456	0.48 (0.29–0.81)†	0.51 (0.30–0.88)*	0.45 (0.26–0.78)†
Quartile 4 (highest)	26/455	0.47 (0.27–0.79)†	0.45 (0.26–0.79)†	0.41 (0.23–0.72)†
p Value for trend		.002	.003	0.001
Animal protein				
Quartile 1 (lowest)	35/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	38/455	0.76 (0.46–1.26)	0.76 (0.45–1.27)	0.68 (0.40–1.17)
Quartile 3	29/456	0.64 (0.38–1.08)	0.61 (0.35–1.04)	0.56 (0.32–0.97)*
Quartile 4 (highest)	30/455	0.56 (0.32–0.97)*	0.52 (0.29–0.93)*	0.48 (0.26–0.87)*
p Value for trend		.030	.019	0.011
Vegetable protein				
Quartile 1 (lowest)	32/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	36/455	0.69 (0.41–1.18)	0.82 (0.46–1.45)	0.76 (0.42–1.37)
Quartile 3	32/456	0.72 (0.42–1.23)	0.95 (0.52–1.74)	0.87 (0.47–1.63)
Quartile 4 (highest)	32/455	0.45 (0.25–0.82)†	0.70 (0.35–1.43)	0.65 (0.31–1.34)
p Value for trend		.002	.003	0.001

Notes: CI = confidence interval; OR = odds ratio.

The *p* values for the OR were obtained using logistic regression: *$p < .05$; †$p < .01$.

Model 1: Logistic regression model adjusted for sex, age (60–69, 70–79, ≥80 years), energy intake (quartiles), ethanol (quartiles), lipids (quartiles), and animal protein or vegetable protein as appropriate.

Model 2: As in Model 1 and additionally adjusted for level of education (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (never, former, current smokers), body mass index (<25, 25–29.9, ≥30 kg/m^2), abdominal obesity (yes, no), and dietary fiber (quartiles).

Model 3: As in Model 2 and additionally adjusted for chronic respiratory disease (yes, no), coronary disease (yes, no), stroke (yes, no), osteoarthritis/arthritis (yes, no), cancer (yes, no), diabetes (yes, no), depression (yes, no), number of medications (continuous), and ex-drinker status (yes, no).

Table 2. ORs (95% CI) of Incident Frailty According to Quartiles of Fatty Acids Intake in the Seniors-ENRICA Cohort Study (*N* = 1,822).

	Frailty cases / *n*	Model 1	Model 2	Model 3
		OR (95% CI)	OR (95% CI)	OR (95% CI)
Monounsaturated fatty acids				
Quartile 1 (lowest)	40/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	29/455	0.77 (0.44–1.33)	0.69 (0.39–1.23)	0.66 (0.37–1.20)
Quartile 3	35/456	0.65 (0.36–1.18)	0.58 (0.31–1.07)	0.54 (0.28–1.02)
Quartile 4 (highest)	28/455	0.59 (0.32–1.10)	0.50 (0.26–0.96)*	0.50 (0.26–0.96)*
p Value for trend		.09	.039	.038
Saturated fatty acids				
Quartile 1 (lowest)	25/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	31/455	1.06 (0.57–0.97)	0.88 (0.47–1.67)	0.90 (0.46–1.74)
Quartile 3	38/456	1.82 (1.00–3.31)	1.49 (0.78–2.85)	1.72 (0.89–3.35)
Quartile 4 (highest)	38/455	1.56 (0.83–2.95)	1.25 (0.62–2.53)	1.28 (0.62–2.63)
p Value for trend		.08	.32	.27
Long-chain ω-3 fatty acids				
Quartile 1 (lowest)	44/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	39/455	0.72 (0.44–1.17)	0.80 (0.48–1.32)	0.72 (0.43–1.21)
Quartile 3	23/456	0.56 (0.32–0.97)*	0.59 (0.33–1.04)	0.62 (0.34.1.12)
Quartile 4 (highest)	26/455	0.68 (0.38–1.22)	0.75 (0.41–1.37)	0.79 (0.43–1.46)
p Value for trend		.09	.18	.30
Alpha-linolenic acid				
Quartile 1 (lowest)	35/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	33/455	0.77 (0.44–1.34)	0.86 (0.48–1.55)	0.87 (0.48–1.58)
Quartile 3	39/456	0.94 (0.53–1.69)	1.04 (0.57–1.90)	1.04 (0.56–1.93)
Quartile 4 (highest)	25/455	0.61 (0.33–1.16)	0.69 (0.36–1.33)	0.71 (0.36–1.39)
p Value for trend		.23	.40	.45
Linoleic acid				
Quartile 1 (lowest)	27/456	1 (Ref)	1 (Ref)	1 (Ref)
Quartile 2	37/455	1.48 (0.81–2.70)	1.56 (0.84–2.90)	1.64 (0.86–3.12)
Quartile 3	41/456	2.32 (1.24–4.33)†	2.38 (1.25–4.54)†	2.29 (1.18–4.45)*
Quartile 4 (highest)	27/455	1.58 (0.81–3.09)	1.84 (0.92–3.67)	1.84 (0.90–3.77)
p Value for trend		.13	.06	.08

Notes: CI = confidence interval; OR = odds ratio.

The *p* values for the OR were obtained using logistic regression: *$p < .05$; †$p < .01$. Models adjusted as in Table 1 including the other fatty acids in the table and total protein intake (quartiles).

Discussion

Total protein, animal protein, and MUFA intakes were inversely associated with incident frailty in community-dwelling older adults. Moreover, higher animal protein intake was associated with lower risk of slow walking speed, and higher MUFA intake was associated with reduced risk of unintentional weight loss. These results were obtained in a sample that was slightly younger than other well-known cohorts in this field, such as the InCHIANTI study (14), and thus the incidence of frailty was somewhat lower. However, our results did not seem to vary with age.

Protein Intake

In addition to the WHI-OS (5), the association of protein intake and frailty has been assessed in three cross-sectional analyses (15–17), with partially consistent results. In Italy, the InCHIANTI study (15) reported that the prevalence of frailty in older adults in the lowest quintile of protein intake was double that of those in the highest quintile. Likewise, in Japanese older women (16), a higher protein intake was associated with lower prevalence of frailty, regardless of the source of protein. Finally, among 194 community-dwelling elders in Germany, the distribution of protein consumption throughout the day, but not the total amount of protein intake, was significantly related to frailty (17). Frail participants showed a different and more uneven distribution of their protein intake during the day, with lower intake at breakfast and higher intake at lunch. However, given the cross-sectional design in these three analyses, frail participants may have changed their diet due to exhaustion, weight loss, or weakness.

In the Health, Aging and Body Composition (Health ABC) study (18), total and animal protein intake, but not vegetable protein, was associated with less lean mass loss in older adults. Those in the highest quintile of protein intake lost 40% less lean mass than did those in the lowest quintile. Similar results were found for appendicular lean mass, which best discriminates the presence of weakness. These results contrast with ours because we did not find an association between protein intake and weakness.

Our results are also of interest because they may shed light on the current discussion on recommended protein intake in older adults. The current recommended dietary allowance (RDA) for protein is 0.8 g/kg/day, and the estimated average requirement is 0.66 g/kg/day. These values apply to all the healthy adult population, including older adults (19). Some studies question whether these recommendations are adequate for this population subgroup, because of the high frequency of significant sarcopenia (19). In our study, the median total protein intake in the third quartile was slightly above the RDA, corresponding to 1.28 g/kg/day (about 95 g/day). Above this value, a higher protein intake was not related to a further decrease in frailty risk. These findings concur with those of the WHI-OS (5) and the

Health ABC study (18), because in both the studies, the highest quintile of protein intake was 1.20 g/kg/day, which is 50% higher than the current RDA (5,18). Moreover, additional evidence supports the recommendation of protein intake averaging 1.20 g/kg/day (20,21), because the threshold for anabolic intake of protein is greater in elderly participants compared with that in young adults. Finally, this amount of protein intake has not been associated with any detrimental effect in the general population (22).

In our study, no association between vegetable protein and frailty was found. This could be because the intake of vegetable protein was lower than that of animal protein, or because the latter is richer in essential amino acids (23) which, in turn, could be the primary inducer of muscle protein anabolism in older adults. Moreover, in a study among normal weight and sedentary women, animal protein was the single predictor of muscle mass index (24).

The observed inverse association between protein intake and slowness is also noteworthy, because slow gait is one of the best predictors of the adverse health outcomes associated with frailty (25). Interestingly, no association was found between protein intake and muscle weakness. Walking is a complex activity, which requires sufficient lean body mass, strength, neurological functioning, and balance, among other functions. Unfortunately, our study only assessed gait speed and strength but not the other variables required for walking.

Overall, our findings support a protein intake of at least 1.2 g/kg/day in older adults, in line with new dietary recommendations. Protein should derive from the main food sources (such as chicken, milk, white fish, matured cheese, beef, ham, white bread), with a preference for those with lower content in saturated fats or salt (26).

MUFA Intake

No previous epidemiologic studies have assessed the role of different types of fats on frailty, although a cross-sectional study in the United Kingdom found that a higher intake of SFAs and MUFAs was associated with a lower fat-free mass in women aged 18 to 79 years (27).

The main sources of MUFAs in Spain and other Mediterranean countries differ from those in northern Europe, America, or Asia. The main source of MUFAs in Spain is olive oil (an essential component of the Mediterranean diet), which is flavorful and often consumed with vegetables, which also have shown a beneficial effect on frailty (28). Other MUFA-rich foods frequently consumed in Spain are red meat, dairy, and pastries. By contrast, the main contributors to total MUFA intake in non-Mediterranean countries are meat and meat products, added fats, and dairy products (29).

Certain mechanisms may account for the relationship between MUFA intake and frailty. The Mediterranean diet (rich in MUFAs) has been associated with lower levels of inflammatory markers (30,31). In addition, these markers are strongly related to frailty (32) and, to some extent, to unintentional weight loss (33). Moreover, extra-virgin olive oil is rich in tocopherols, which provide oxidative stability and have scavenging activity (34). Tocopherols and tocotrienols protect lipids from peroxidation due to their complex organization in the phospholipid bilayers, and α-tocopherol spontaneously associates with unsaturated fatty acids (35,36).

We found a strong inverse association between MUFA intake and unintentional weight loss. Although the mechanisms of this association are uncertain, there is some evidence that a high-MUFA diet versus a high-SFA diet may help in maintaining body weight, energy balance, and insulin sensitivity (37). Moreover, fatty acids produce a lower satiating effect than the same isocaloric quantity of dietary carbohydrate (38), which could prevent unintentional weight loss.

Overall, our results suggest that intake of MUFAs from olive oil, as consumed in the traditional Mediterranean diet, could contribute to frailty prevention.

Strengths and Limitations

The strengths of this study include its prospective design and the adjustment for many potential confounders. Among the limitations was the relatively small number of frailty cases, which nevertheless did not impede observation of several significant associations. Also, diet was self-reported and a certain recall bias cannot be ruled out. Finally, although animal protein and MUFA intake were associated with incident frailty after adjustment for an extensive number of potential confounders, some residual confounding cannot be dismissed.

In conclusion, in this prospective study of older adults in Spain, a higher intake of total and animal protein and MUFAs was associated with lower risk of frailty. These findings add to the evidence base for the development of recommendations on food and nutrient intake in older adults.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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Conflict of Interest

None.

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Protein Intake and Risk of Falls: A Prospective Analysis in Older Adults

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BACKGROUND: The prospective association between protein intake and falls has been little studied. We assessed this association in a Spanish community-dwelling cohort.

METHODS: We performed a prospective cohort study of 2464 men and women 60 years or older who were recruited in 2008-2010 and followed up through 2012. At baseline, the habitual protein intake was determined with a validated dietary history. At the end of follow-up, participants reported the number of falls experienced in the preceding year. Participants were stratified by an unintentional weight loss of 4.5 kg or more. Logistic regression was used after adjustment for the main confounders.

RESULTS: A total of 522 participants (21.2%) experienced at least one fall. The odds ratios (ORs) and 95% confidence intervals (CIs) of falling for the three increasing tertiles of total protein intake were 1.00, 0.86 (0.66-1.11), and 0.93 (0.70-1.24) (p for trend = 0.14). However, a statistically significant interaction with unintentional weight loss was observed for the association between protein intake and fall risk (p for interaction = 0.004). Among 163 participants (6.6%) who experienced unintentional weight loss, the ORs (95% CI) of falling for the three increasing tertiles of total protein intake were 1.00, 0.68 (0.21-2.23), and 0.23 (0.05-1.08) (p for trend = 0.01).

CONCLUSION: No protective association between protein intake and fall risk in older adults was found. However, high total protein intake tended to confer substantial benefits to participants who experienced an unintentional weight loss of 4.5 kg or more in the preceding year. J Am Geriatr Soc 00:1–7, 2018.

Key words: falls; protein intake; animal protein intake; vegetable protein intake; unintentional weight loss; older adults

INTRODUCTION

Falls are a major public health concern for the older adult population.[1–3] Several epidemiological studies have identified medical risk factors for falling among older adults, such as age, depression, polypharmacy, sleeping medications, and hypotension.[4,5] However, the prevention of falls among the elderly is complex,[5] and potential dietary determinants are only poorly identified.[6]

Previous results from the Framingham Study[7] showed no association between protein intake and falls. However, the Framingham Study showed a protective association among those who had lost weight. Moreover, in the Study of Osteoporotic Fractures,[8] neither dietary protein intake nor vitamin D was associated with a decreased risk of falls. In addition, there is no conclusive information about the role of protein intake and the risk of hip fracture in older adults.[9,10]

The main objective of this study was to assess the prospective association between total protein intake and the risk of falling in a community-dwelling population of older adults from Spain. Secondary objectives were to assess the association of animal protein and vegetable protein intake with the risk of falling in the same population and to summarize the findings from the literature. Furthermore, the analysis was stratified according to participants who experienced unintentional weight loss.

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METHODS

Study Design and Population

The data were collected for the Seniors-ENRICA study that focused on a cohort of noninstitutionalized individuals 60 years and older in Spain. The cohort was derived from the ENRICA study, a survey conducted in 2008-2010 that was representative of the noninstitutionalized adult population in Spain.[11,12] ENRICA study participants 60 years or older comprised the Seniors-ENRICA cohort (n = 3289). At baseline, data were collected in three stages. First, a computer-assisted telephone interview was performed to obtain information on sociodemographic factors, health behaviors, and morbidity. Second, a home visit was made to collect blood and urine samples. Third, a second home visit was made to obtain a dietary history and prescribed medications; a physical examination was also performed during this visit. Participants were followed up until 2012 (mean follow-up time of 3.5 y) when a second wave of data collection was performed. Of the initial sample, 675 participants were lost to follow-up, 95 died, 18 were excluded for a lack of data related to falls, 36 were excluded for a lack of data on potential confounders, and 1 was excluded for a lack of reliable dietary information data. The final analyses were performed with 2464 subjects. The baseline sociodemographic, lifestyle, and clinical characteristics of the participants included in the analyses were similar to those of the participants lost to follow-up (data not shown).[11]

The study participants gave written informed consent. The Clinical Research Ethics Committee of La Paz University Hospital in Madrid approved the study.

Diet

Information on diet was collected using a computerized dietary history (HD-ENRICA) that collects habitual dietary consumption practices during the previous year with questions related to all possible meals throughout the day. The history first asks about the food eaten upon arising in the morning and ends with asking about the food consumed before going to bed. Questions were asked about the food consumed in a typical week, and all foods consumed at least once every 15 days were recorded. In addition, information on seasonal and weekend food consumption was obtained. The data were collected by certified interviewers. The protein consumption collected with this instrument was validated against seven 24-hour recalls in the preceding year, and high correlation coefficients were obtained (total protein $\rho = 0.59$; animal protein $\rho = 0.62$; vegetable protein $\rho = 0.62$).[13] The intake of total energy, total protein, animal protein, and vegetable protein, as well as the intake of caffeine, calcium, vitamin D, and alcohol, was estimated using standard food composition tables from Spain.[14,15]

Falls

A fall was defined as "an event which resulted in a person coming to rest inadvertently on the ground or floor or other lower level."[16] At the end of follow-up, participants reported the number of falls experienced in the preceding year by answering this question: "How many times did you fall during the past year?" The responses were recorded on a continuum from zero (no falls) to nine or more. Participants who had fallen were asked this question: "As a consequence of the fall, did you need to go to the doctor, go to the emergency room, or be hospitalized, or did you not need medical attention?" A participant was deemed to have required medical attention when the answer to any of the first three options was "yes."

Other Variables

At baseline, information on sex, age, educational level, smoking status, and alcohol consumption was obtained. Weight and height were measured under standardized conditions,[17,18] and the body mass index (weight in kilograms divided by height in meters squared [kg/m^2]) was calculated. In addition, participants reported their physical activity during leisure activities and at home with the European Prospective Investigation into Cancer and Nutrition cohort questionnaire.[17] The time devoted to each activity was transformed into metabolic equivalent (MET)-hours/week, and the MET-hours/week from the different activities were summed. Participants also self-reported the time spent sleeping during the night or during the day, as well as the time devoted to watching TV. Participants were also asked whether they had different chronic conditions such as cardiovascular diseases, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, hip fracture, cancer, and/or depression requiring medication. The number of daily medications was checked against medication containers, and participants reported whether they usually took medications for sleeping or hypertension. The quality of the diet was estimated by the Mediterranean Diet Adherence Screener without considering wine consumption.[19] Finally, participants reported whether they lived alone, and we inquired about their dependence in performing instrumental activities of daily living (IADLs) using the Lawton and Brody questionnaire.[20]

In 2012, unintentional weight loss was self-reported. Every participant was asked if he or she had experienced an involuntary weight loss of 4.5 kg (10 lb) or more in the preceding year, in accordance with the standardized definition developed by Fried et al.[21]

Statistical Analysis

Logistic models were used to assess the risk of falls. Protein intake was classified into sex-specific tertiles, and the lowest tertile was used as the reference. The models were adjusted not only for socioeconomic variables, lifestyle, diseases, and nonprotein dietary components but also for other variables associated with falls in the literature.[22]

Logistic models were established to adjust for sex; age (continuous); educational level (primary or less, secondary, or university); smoking status (never-smoker, ex-smoker, or smoker); alcohol intake status (never-drinker, occasional drinker, usual drinker, or ex-drinker); body mass index in kg/m^2 (≤ 25, >25 to <30, or ≥ 30); physical activity in MET-hours/week (continuous); time spent sleeping (continuous); time spent watching TV (continuous); presence of cardiovascular disease, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, history of hip fracture,

cancer, and/or depression requiring medication; number of medications (continuous); use of sleeping or hypertension medications; intake of energy, caffeine, calcium, and vitamin D; adherence to a Mediterranean diet (continuous); living alone; dependence in performing IADLs, and intake of animal protein (quartiles) or vegetable protein (quartiles) as appropriate. The *p* for trend was calculated by treating the tertiles as a continuous variable. Interactions between the association of interest with both unintentional weight loss and age were assessed using the likelihood ratio test, and models were compared with and without the interaction terms.

The adjusted predictive risk of falls according to unintentional weight loss status (no weight loss or weight loss) was plotted against the total protein intake considered as a continuous variable. Statistical significance was set at a two-sided $p < 0.05$. The analyses were conducted with Stata/SE, v.14.1 (StataCorp, College Station, TX).

RESULTS

The final sample comprised 2464 participants (53.0% female) with a mean age of 69 years at baseline. After a mean follow-up of 3.5 years, a total of 522 participants (21.2%) had fallen at least once in the preceding year. The means for protein consumption were as follows: 92 g/d (standard deviation [SD] = 26 g/d) for total protein, 61 g/d (SD = 22 g/d) for animal protein, and 31 g/d (SD = 10 g/d) for vegetable protein.

Participants consuming more total protein and animal protein were younger, less frequently ex-drinkers, heavier, taller, and more frequently obese; suffered from cancer more frequently; had higher consumption of energy, calcium, and vitamin D; followed a Mediterranean diet more frequently, lived alone less frequently; and had less dependence in performing IADLs than those consuming less total protein and animal protein. However, those who consumed more vegetable protein had similar ages, weighed less, were more physically active, had cancer less frequently, and did not consume a greater amount of vitamin D than those who consumed less vegetable protein (Table 1).

Supplementary Table S1 presents the crude results for the risk of falling. The adjusted odd ratios (ORs) and 95% confidence intervals (CIs) of falling for the three increasing tertiles of total protein intake were 1.00, 0.86 (0.66-1.11), and 0.93 (0.70-1.24) (*p* for trend = 0.14). Thus when the association between total protein intake and having experienced at least one fall was assessed, the estimates were less than 1 in the fully adjusted model, but none of the associations were statistically significant. Similar results were obtained for animal and vegetable protein intake.

However, a statistically significant interaction was observed with unintentional weight loss when the association between protein intake and risk of falls was assessed (*p* for interaction = 0.004). For participants who lost 4.5 kg or more in the preceding year, the adjusted ORs and 95% CIs of falling for the three increasing tertiles of total protein intake were 1.00, 0.68 (0.21-2.23), and 0.23 (0.05-1.08) (*p* for trend = 0.01) (Table 2). In all cases, the estimators decreased linearly, but the interactions were statistically significant only for vegetable protein intake when those in the highest consumption tertile were compared with those in the lowest consumption tertile (OR = 0.20; 95% CI = 0.05-0.84). The interaction was statistically significant in all analyses (Table 2 and Figure 1).

We performed sensitivity analyses to assess the robustness of the main results. We obtained similar results when excluding participants with a history of hip fracture, when reestimating the study association using different methodologies to adjust for energy consumption, when adjusting for ethanol (g/d), and when excluding Mediterranean diet as a confounder. When falls requiring medical attention were used as an end point, the results exhibited a trend similar to those of the other analyses, but the associations were not statistically significant due to a lack of events. Finally, we did not find a statistically significant interaction between protein intake and age.

DISCUSSION

No protective association was found between protein intake and the risk of falling in older adults in this community-dwelling cohort of older adults. However, when the association between protein intake and fall risk was assessed, a statistically significant interaction was observed with unintentional weight loss. An inverse trend was observed between protein intake and the risk of falls in participants who had a weight loss of 4.5 kg or more in the preceding year.

The association between protein intake and falls has been little studied. However, a cross-sectional study conducted with 100 healthy volunteers found that the protein intake of fallers and nonfallers was similar.[23]

Concerning longitudinal studies, our results are in line with those from the Framingham Study[7] that included 807 participants with a mean age of 75 years, in showing no association between protein intake and falls. However, in the Framingham Study, the results for the participants who had lost weight were statistically significant and more extreme than the results in our study. Compared with our sample, the sample in the Framingham Study was slightly older and had a lower protein intake.

In the Study of Osteoporotic Fractures,[8] neither dietary protein intake nor vitamin D intake was associated with falls. This study comprised more than 4000 very old postmenopausal women with a relatively low protein intake. Furthermore, neither dietary protein intake nor vitamin D was associated with falls after adjusting for potential confounders.

Hip fracture was used as the outcome in two cohort studies.[9,10] In both studies, the number of events was small, and the estimates were less than 1 but not statistically significant. However, these results are not totally comparable with those in our study. Higher protein intake was related to an increase in urinary calcium that may lead to osteoporosis.[24] Additionally, other studies found that higher protein intake is associated with an increased intestinal absorption of calcium,[25] suggesting the higher urinary calcium observed indicates calcium absorption and not bone resorption. Associations of protein intake with falls could have determinants different from those for associations of protein intake with fractures, thus requiring further studies with a larger sample size.

Table 1. Baseline characteristics of the study participants[a]

	Total protein intake				Animal protein intake				Vegetable protein intake			
	T1	T2	T3	*P* for trend	T1	T2	T3	*P* for trend	T1	T2	T3	*P* for trend
Age, y, mean (SD)	69.5 (6.7)	68.5 (6.4)	67.9 (5.9)	<.001	69.3 (6.6)	68.6 (6.5)	68.0 (5.9)	<.001	68.7 (6.5)	68.6 (6.2)	68.6 (6.4)	.71
Educational level, %												
Primary or less	56.3	53.9	53.7	.48	55.7	55.6	52.6	0.57	52.1	54.0	57.8	.19
Secondary	22.1	25.8	24.6		22.9	24.5	25.2		25.4	24.0	23.2	
University	21.5	20.3	21.7		21.4	20.0	22.2		22.5	22.0	19.0	
Smoking status, %												
Never-smoker	59.5	59.5	57.4	.69	60.6	57.8	58.1	.79	57.7	58.8	60.0	.48
Ex-smoker	28.8	30.3	30.5		28.5	30.5	30.6		29.6	29.9	30.1	
Smoker	11.7	10.2	12.1		10.9	11.7	11.3		12.8	11.3	9.9	
Alcohol consumption, %												
Never-drinker	33.7	34.6	38.2	.05	34.4	34.1	37.9	.13	36.7	33.6	36.1	<.001
Occasional drinker	9.3	7.9	7.4		9.5	8.5	6.6		5.6	9.9	9.6	
Usual drinker	46.6	49.3	47.9		47.2	48.1	48.5		46.0	48.1	49.7	
Ex-drinker	10.5	8.3	6.5		8.9	9.4	7.0		11.7	8.5	5.0	
Weight, kg, mean (SD)	73.0 (12.4)	73.5 (13.1)	76.1 (13.4)	<.001	72.5 (12.4)	74.0 (12.8)	76.1 (13.6)	<.001	74.9 (13.4)	74.3 (12.8)	73.3 (12.8)	.009
Height, cm, mean (SD)	160.3 (8.8)	161.3 (9.2)	162.0 (8.9)	<.001	160.6 (8.8)	161.1 (8.8)	161.8 (9.3)	.005	160.8 (9.4)	161.5 (8.8)	161.3 (8.7)	.22
Body mass index												
≤25 kg/m^2	18.0	22.6	17.4	.005	21.4	18.5	18.2	.003	15.8	20.2	22.1	.003
>25 to < 30 kg/m^2	52.0	47.9	47.1		51.7	49.4	45.9		48.4	49.3	49.3	
≥30 kg/m^2	30.1	29.4	35.5		26.9	32.1	36.0		35.8	30.5	28.7	
Physical activity, MET-h/week, mean (SD)	57.5 (34.0)	61.0 (34.3)	60.2 (34.4)	.11	59.2 (34.2)	60.2 (35.0)	59.2 (34.6)	.94	55.6 (33.1)	61.2 (35.4)	61.9 (34.9)	<.001
Time sleeping, h, mean (SD)	7.2 (1.6)	7.1 (1.6)	7.2 (1.5)	.53	7.1 (1.5)	7.2 (1.5)	7.1 (1.5)	.99	7.1 (1.5)	7.2 (1.4)	7.1 (1.5)	.81
Time watching TV, h, mean (SD)	2.6 (1.7)	17.8 (10.9)	2.6 (1.4)	.89	2.6 (1.7)	17.8 (10.6)	2.6 (1.6)	.69	2.6 (1.7)	17.9 (10.9)	2.6 (1.4)	.99
Cardiovascular disease, %	5.2	5.5	5.7	.91	5.7	5.7	5.0	.76	5.0	6.3	5.1	.42
Hypertension, %	65.2	63.9	65.5	.76	65.3	63.8	65.5	.71	66.8	64.5	63.3	.32
Diabetes, %	16.4	13.9	16.5	.98	14.7	16.6	15.5	.59	15.9	16.3	14.5	.57
Chronic pulmonary disease, %	7.5	6.8	7.3	.84	8.0	6.3	7.3	.41	7.5	6.2	7.9	.37
Arthritis or osteoarthritis, %	46.2	49.8	50.0	.23	47.0	49.8	49.2	.48	47.6	48.1	50.4	.48
History of hip fracture, %	1.7	1.0	1.5	.67	1.7	1.5	1.0	.21	1.8	0.6	1.7	.83
Cancer, %	1.2	1.7	2.9	.01	1.1	2.1	2.7	.02	2.0	2.0	2.0	.99
Depression requiring medication, %	8.6	8.3	7.9	.87	8.9	7.9	8.1	.74	8.5	8.3	8.1	.94
No. of medications, mean (SD)	2.1 (1.9)	1.9 (1.9)	2.0 (2.0)	.36	2.0 (1.9)	2.0 (1.9)	2.0 (2.0)	.55	2.0 (1.9)	1.9 (1.9)	2.0 (2.0)	.60
Sleeping treatment, %	20.1	20.9	19.5	.77	21.6	19.2	19.6	.42	21.4	19.2	19.8	.52
Hypertension treatment, %	38.6	38.8	37.2	.77	39.1	37.8	37.7	.82	40.1	37.7	36.7	.34
Energy, kcal/d, mean (SD)	1843 (1036)	2053 (514)	2,262 (560)	<.001	1963 (1039)	2033 (549)	2,162 (582)	<.001	1861 (1060)	2045 (519)	2,252 (521)	<.001
Caffeine intake, mg/d, mean (SD)	69.0 (119)	74.7 (110)	70.1 (103)	.84	70.1 (123)	73.2 (123)	70.5 (101)	.96	69.5 (114)	74.5 (110)	69.8 (110)	.96
Calcium intake, mg/d, mean (SD)	714 (244)	891.4 (254)	1,058 (428)	<.001	729 (237)	884.6 (261)	1,049 (439)	<.001	872 (441)	884.4 (307)	907 (279)	.04
Vitamin D, µ/d, mean (SD)	2.4 (2.2)	3.4 (2.5)	4.6 (3.9)	<.001	2.5 (2.0)	3.4 (2.6)	4.5 (4.1)	<.001	3.4 (3.7)	3.4 (2.8)	3.5 (2.7)	.53
Mediterranean diet, MEDAS, mean (SD)	6.6 (1.7)	7.0 (1.8)	7.2 (1.7)	<.001	6.7 (1.9)	6.9 (1.7)	7.1 (1.6)	<.001	6.7 (1.5)	6.9 (1.7)	7.3 (1.9)	<.001
Living alone, %	20.2	15.1	14.8	.004	19.8	13.3	17.0	.002	19.2	17.5	13.3	.004
Dependence in performing IADLs, %	13.1	8.5	10.0	.008	12.4	9.7	9.5	.10	11.9	10.0	9.8	.29

[a]According to tertiles of total protein, animal protein, and vegetable protein intake in the Seniors-ENRICA cohort study (N = 2464).

Note: Cutoff points for protein intake tertiles (in g/d): for men, total protein: T1, 18.4-87.9; T2, 88.0-108.5; T3, 108.6-284.7; animal protein: T1, 10.3-55.7; T2, 55.8-74.5; T3, 74.6-260.2; vegetable protein: T1, 8.1-28.5; T2, 28.6-37.1; T3, 37.2-79; for women, total protein: T1, 26.8-72.8; T2, 72.9-90.7, T3, 90.8-207.1; animal protein: T1, 11.3-45.7; T2, 45.8-61.2; T3, 61.3-374.5; vegetable protein: T1, 1.6-23.4; T2, 23.5-31.1; T3, 31.2-88.4.

Abbreviations: IADLs = instrumental activities of daily living; MEDAS = Mediterranean Diet Adherence Screener without alcohol intake; MET = Metabolic equivalent; SD = standard deviation; T1 = tertile 1 (lowest); T3 = tertile 3 (highest).

The association of protein intake with falls among individuals with unintentional weight loss could be related to frailty. Frailty is an age-associated syndrome that includes sarcopenia as its pathophysiologic basis,[26] and unintentional weight loss, evaluated as the involuntary loss of 4.5 kg or more in the preceding year, is one of the frailty criteria in the definition developed by Fried et al.[21] Another frailty criterion is muscle weakness, defined as classification in the lowest quintile of grip strength within groups based on sex and body mass index in our cohort. In addition, the daily consumption of at least 1 g/kg/d protein is associated with a decreased loss of knee strength that might be an additional mechanism by which protein intake protects against falls.[27] Evidence suggests that interventions to

Table 2. Odds ratios and incidence rate ratios (95% confidence intervals) for the risk of falls[a]

	All participants		Participants without weight loss		Participants with weight loss (≥4.5 kg)		
	Events/n 522/2464	OR (95% CI)	Events/n 477/2301	OR (95% CI)	Events/n 45/163	OR (95% CI)	*P* for interaction
Total protein							.004
Tertile 1, lowest	184/822	1 (ref.)	164/767	1 (ref.)	20/55	1 (ref.)	
Tertile 2	164/822	0.86 (0.66-1.11)	147/762	0.86 (0.66-1.23)	17/60	0.68 (0.21-2.23)	
Tertile 3, highest	174/820	0.93 (0.70-1.24)	166/772	0.98 (0.73-1.31)	8/48	0.23 (0.05-1.08)	
P for trend		0.14		0.35		0.01	
Animal protein							.017
Tertile 1, lowest	189/822	1 (ref.)	169/765	1 (ref.)	20/57	1 (ref.)	
Tertile 2	156/822	0.81 (0.62-1.06)	140/760	0.81 (0.62-1.06)	16/62	0.65 (0.19-2.24)	
Tertile 3, highest	177/820	0.96 (0.71-1.28)	168/776	0.96 (0.72-1.28)	9/44	0.29 (0.07-1.28)	
P for trend		0.13		0.24		0.02	
Vegetable protein							.016
Tertile 1, lowest	190/822	1 (ref.)	169/772	1 (ref.)	21/50	1 (ref.)	
Tertile 2	160/822	0.86 (0.66-1.12)	149/771	0.86 (0.66-1.12)	11/51	0.20 (0.05-0.74)	
Tertile 3, highest	172/820	0.91 (0.70-1.20)	159/758	0.91 (0.70-1.20)	13/62	0.20 (0.05-0.84)	
P for trend		0.38		0.68		0.02	

[a]According to tertiles of protein intake in the Seniors-ENRICA cohort study (N = 2464). Analyses were stratified by a weight loss ≥4.5 kg in the preceding year.

Note: Models were adjusted for sex, age, educational level, smoking status, alcohol intake status, body mass index, physical activity, time sleeping, time watching TV, cardiovascular disease, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, history of hip fracture, cancer, and/or depression (requiring medication), number of medications, sleeping treatment, hypertension treatment, energy intake, caffeine intake, calcium intake, vitamin D intake, Mediterranean diet, living alone, and dependence in performing instrumental activities of daily living. Animal protein and vegetable protein intake were adjusted for each other as appropriate.

Abbreviations: CI = confidence interval; OR = odds ratio.

improve dietary protein intake may prevent the development of sarcopenia and frailty.[28,29] Furthermore, a systematic review and meta-analysis associated frailty with the risk of falls,[30] and protein intake was related to a lower risk of frailty in the WHI-OS study.[31] Additionally, in a previous analysis of this cohort,[29] the intake of protein and monounsaturated fatty acids was strongly and inversely associated with frailty and more specifically with slow walking, considered a predictor of falls.[32] Similarly, Rahi et al found that a daily protein intake of 1 g/kg/d was associated with a lower prevalence of frailty in community-dwelling older adults.[33]

In the NuAge study,[34] a protein intake of more than 1.0 g/kg/d was found to protect against a 1-year weight loss of 5% or more. Additionally, protein intake may attenuate not only the age-related loss of skeletal muscle but also the development of sarcopenia,[35] an independent predictor of incident falls.[36,37] Interestingly, in participants in the Health ABC study who lost weight, higher protein intake was related to lower lean muscle loss, whereas in those with a stable weight, no association was found.[35] Similarly, some studies related unintentional weight loss to an increased risk of frailty fractures and falls.[38,39] Thus two of the frailty criteria, namely unintentional weight loss and muscle strength, were associated with protein intake. Moreover, frailty was associated with the risk of falls.[30]

Finally, laboratory analyses showed that some proteins may influence unintentional weight loss in older humans; some of these proteins (such as neuropeptide Y and peptide YY) are altered in both aging animals and aging humans.[40] Therefore, our results are consistent with existing biological and medical knowledge and emphasize the important role of dietary protein intake on sarcopenia prevention and muscle protein synthesis[28] mediated or not mediated by frailty.

Some authors suggest increasing the recommendations for the dietary allowance of protein that currently is 0.8 g/kg/d.[41] Additionally, a randomized controlled trial performed with malnourished older adults showed that an intervention consisting of a protein-enriched diet, calcium vitamin supplements, and telephone counseling by a dietitian decreased the number of patients who suffered falls,[42] emphasizing the protective effect of protein consumption in malnourished older adults.

Our results may be relevant in older adults because protein intake might decrease the risk of frailty syndrome and falls among individuals who lose weight. Of special interest are proteins from vegetables, since the consumption of fruit and vegetables was also associated with a reduced risk of frailty,[43] and fruits and vegetables seem to be especially beneficial for individuals who have lost weight.

A strength of this study is its prospective design. Additionally, the study results were robust because they did not change substantially across many sensitivity analyses. A relatively large number of events were included, and participants were an unselected sample of free-living community residents. However, limitations of the study should be noted. As in most nutritional epidemiology studies, diet was self-reported. The HD-ENRICA data were validated against seven 24-hour recalls collected every 2 months during the previous year.[13] However, recall bias cannot be totally ruled out when questionnaires are used to assess habitual dietary consumption. Additionally, the underreporting of falls is possible because of recall bias, thus leading to a misclassification bias toward the null value. Additionally, residual confounding cannot be ruled out because of the observational design. Finally, a survival bias could be present because hip fracture increases mortality, and participants lost to follow-up could be more likely to have

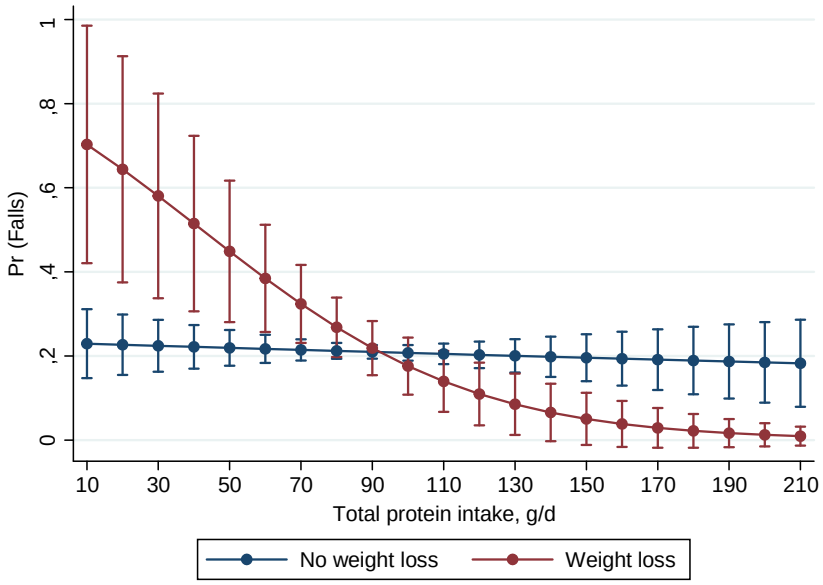

Figure 1. Adjusted predictive risk of falls with 95% confidence intervals, according to an unintentional weight loss of 4.5 kg or more in the preceding year, from the Seniors-ENRICA cohort study (N = 2464). Models were adjusted for sex, age, educational level, smoking status, alcohol intake status, body mass index, physical activity, time sleeping, time watching TV, cardiovascular disease, hypertension, diabetes, chronic pulmonary disease, arthritis or osteoarthritis, history of hip fracture, cancer, and/or depression (requiring medications), number of medications, sleeping treatment, hypertension treatment, energy intake, caffeine intake, calcium intake, vitamin D intake, Mediterranean diet, living alone, and dependence in performing instrumental activities of daily living.

experienced weight loss and falls. However, the effect of this bias is probably small because hip fracture occurs in only approximately 1% of falls,[44] and the baseline characteristics of the participants included in the analyses were similar to those of the participants lost to follow-up.

In conclusion, no protective association was found between protein intake and the risk of falling in older adults. However, participants who had experienced an unintentional weight loss of 4.5 kg or more in the preceding year benefited substantially from high protein intake. Consequently, a broad and integrative approach is needed to address the association between protein intake and fall prevention in older adults. Further studies should consider the influence of weight loss on this association.

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Conflicts of Interest: None.

Author Contributions: Helena Sandoval-Insausti and Pilar Guallar-Castillón conceived the article. Helena Sandoval-Insausti and Raúl F. Pérez-Tasigchana performed the analyses. Esther López-García, José R. Banegas, and Fernando Rodríguez-Artalejo made important contributions. All the authors approved the final text of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Supplementary Table S1. Unadjusted odds ratios (95% confidence interval) for the risk of falls according to tertiles of protein intake in the Seniors-ENRICA cohort study (N = 2464). Analyses were stratified by a weight loss of ≥4.5 kg in the preceding year.

Ultra-processed Food Consumption and Incident Frailty: A prospective Cohort Study of Older Adults

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Short running head: Ultra-processed food consumption and frailty.
Key words: Ultra-processed foods, frailty, older adults.

Brief summary: The consumption of ultra-processed foods is strongly associated with frailty risk in older adults, adding evidence for the restriction of ultra-processed foods and the promotion of fresh or minimally processed foods.

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European Prospective Investigation into Cancer and Nutrition, EPIC
body mass index, BMI
odds ratios, ORs
confidence interval, CI
gram per day/body weight, g/Kg

DECLARATIONS

Ethics approval and consent to participate: Study participants gave written informed consent. The Clinical Research Ethics Committee of "La Paz" University Hospital in Madrid (Spain) approved both, the baseline and follow-up studies.
Consent for publication: Not applicable.
Availability of data and material: The datasets analyzed during the current study are not publicly available. However, it can be made available from the corresponding author on reasonable request.
Competing interests: None.
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Authors' contributions: HS-I and PG-C conceived the article; HS-I, RB-R, and PG-C performed statistical analyses; HS-I and PG-C wrote the article; AG, EL-G, BM-F, ML, CD-V, JMO, and FR-A made important contributions; and all authors approved the final text of the manuscript.

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ABSTRACT

Background and Objective: Ultra-processed food intake has been associated with chronic conditions. The aim of this study was to assess the relationship between ultra-processed food intake and incident frailty in community-dwelling older adults.

Design: Prospective cohort study with 1,822 individuals aged ≥60 who were recruited during 2008-2010 in Spain. At baseline, food consumption was obtained using a validated computerized face-to-face dietary history. Ultra-processed foods were identified according to the nature and extent of their industrial processing (NOVA classification). In 2012, incident frailty was ascertained based on Fried's criteria. Statistical analyses were performed with logistic regression and adjusted for the main potential confounders.

Results: After a mean follow-up of 3.5 years, 132 cases of frailty were identified. The odds ratios (95% confidence interval) for frailty across increasing quartiles of the percentage of total energy intake from ultra-processed foods were: 1.00, 1.52 (0.78-2.96), 2.98 (1.62-5.50), and 3.67 (2.00-6.73); *p* for linear trend: <0.001. Results were similar when food consumption was expressed as gram per day/body weight. Regarding ultra-processed food groups, the highest versus the lowest tertiles of consumption of yogurts and fermented milks, cakes and pastries, as well as non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks) were also significantly related to incident frailty.

Conclusions and implications: Consumption of ultra-processed foods is strongly associated with frailty risk in older adults.

Key words: Ultra-processed foods, frailty, older adults.

Introduction

Frailty syndrome is characterized by a loss of functional reserve, and sarcopenia is its most important pathophysiological substrate. Frail older adults have an increased vulnerability even to minor stressors (such as having a fever, a urinary tract infection), and it has been linked to a rise in institutionalization and premature death.(1-3) Frailty is a potentially reversible syndrome through physical activity and a high quality diet.

Dietary factors have been related to frailty and disability.(4, 5) The risk of frailty is inversely associated with total protein, animal protein, and monounsaturated fatty acids intake.(6) Also, the intake of antioxidants, B-vitamins,(7-9) and the adherence to a Mediterranean dietary pattern(10, 11) have been related to a lower incidence of frailty. On the other hand, mixed results have been obtained for the consumption of dairy products.(12)

Ultra-processed foods are defined as food or drink products formulated mostly or entirely from substances derived from foods and additives, with little if any unaltered food.(13, 14) These foods include extracted or refined oils, hydrogenated oils and fats, protein isolates, different types of flour and starches, sugar variants, and remnants of animal foods.(15-17) They usually have a high content of total fat, saturated fat, trans fat, simple sugars, and salt, but a low content of proteins, fiber, and vitamin density.(14, 18-20)

Ultra-processed foods are durable, palatable, ready to eat, drink, or heat, and mostly affordable.(16, 20) After aggressive marketing,(21) their consumption is growing

substantially even in middle- and low- income countries.(15) There is evidence that ultra-processed food consumption is associated with an increased risk of chronic diseases and metabolic risk factors, such as overweight/obesity,(22, 23) hypertension,(24) high total cholesterol and low-density lipoprotein cholesterol levels in children,(25) metabolic syndrome in adolescences,(26) cancer,(27) and other chronic diseases.(28)

Although a recent paper analyzing data from the Senior-ENRICA cohort found that the consumption of added sugars in the diet of older people was associated with frailty, and mainly when present in processed foods,(29) to our knowledge, no previous epidemiological studies have comprehensively assessed the association of ultra-processed food intake with frailty.

Methods

Study Design and Population

Data were taken from the Seniors-ENRICA (Study on Nutrition and Cardiovascular risk factors in Spain) cohort. In brief, the cohort was established in 2008-2010 with 2,614 individuals of the community-dwelling population aged 60 years and older in Spain.(30, 31) At baseline, a computer-assisted phone interview was performed to obtain information on sociodemographic factors, lifestyle, and morbidity. Additionally, two home visits were performed to collect blood and urine samples, to obtain a dietary history, and to conduct a physical examination. Participants were followed up until 2012 (mean follow-up time of 3.5 years), when a second wave of

data collection was performed. From the initial sample, 434 participants were lost, and 95 died during follow-up. Among survivors, 174 were excluded for being frail or a lack of data on frailty at baseline, 81 were excluded for a lack of data on potential confounders, and 8 were excluded for a lack of reliable dietary information data. Finally, analyses were performed with 1,822 participants. The sociodemographic, lifestyle, and clinical characteristics at baseline were similar in those who provided updated information in 2012 and those who did not (data not shown). Study participants gave written informed consent. The Clinical Research Ethics Committee of "La Paz" University Hospital in Madrid (Spain) approved both, the baseline and follow-up studies.

Study Variables

Diet

Habitual food consumption was assessed with a validated computerized face-to-face dietary history (DH-ENRICA) developed from that used in the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort study in Spain.(32, 33) The dietary history included all habitual foods consumed at least once every 15 days. During the interview, the information was summarized in a typical week representing the previous year. DH-ENRICA allowed registering a wide amount of food items and 30 different cooking methods, and used a set of more than 120 photographs to help in estimating the amount of consumption.

All recorded foods were classified according to NOVA based on the extent and purpose of industrial food processing.(14) The justification of NOVA food definition and classification has been reported elsewhere.(13, 18, 34) In brief, the NOVA classification includes four groups: *1)* unprocessed or minimally processed foods: fruit and vegetables, grains, nuts and seeds, fresh and pasteurized milk, and natural yogurt with no added sugars or artificial sweeteners; *2)* processed culinary ingredients: salt, sugar, honey, vegetable oils, butter, lard, and vinegar; *3)* processed foods: canned or bottled vegetables and legumes, fruits in syrup, canned fish, unpackaged cheeses, freshly made bread, and salted or sugared nuts and seeds; and *4)* ultra-processed foods. Ultra-processed foods are those formulated mostly or entirely from food-derived substances. Some examples of ultra-processed foods are burgers, frozen pizza and pasta dishes, nuggets and fish fingers, crisps, mass-manufactured breads and cakes, cookies, jams and confectionery, margarines, cereal bars, carbonated and other sugary drinks, sugary or sweetened milks, and various snack products.(15-17, 24, 35) Food items according to NOVA classification are shown in Supplementary Table 1. The intake of total energy from ultra-processed foods was estimated using standard food composition tables from Spain.(33)

Frailty

A slight modification of the frailty definition proposed by Fried et al in the Cardiovascular Health Study was used.(1) Specifically, frailty was defined as having three or more of the following five phenotypic criteria as follows. *1)* Exhaustion, evaluated as responding to "3 or more days a week" to at least one of the following

questions taken from the Center for Epidemiologic Studies-Depression Scale(36): "I felt that anything I did was a big effort" or "I felt that I could not keep on doing things." *2)* Muscle weakness, defined as the lowest quintile of maximum grip strength on the dominant hand, adjusted for sex and body mass index (BMI) in our cohort. Strength was measured with a Jamar dynamometer, and we selected the highest value in two consecutive measurements.(37, 38) *3)* Low physical activity, defined as walking 2.5 hours per week or less in men and 2 hours per week or less in women (corresponding to the lowest quintiles in our cohort). *4)* Slow walking speed, considered as the lowest quintile in our study sample for the three-meter walking speed test, adjusted for sex and height.(38, 39) *5)* Unintentional weight loss, when 4.5 kg (10 lb) or more of body weight was lost in the preceding year.

Other variables

At baseline, data on sociodemographics, lifestyles, and morbidities were collected. Self-reported information was obtained on sex, age, marital status, educational level, tobacco consumption, ex-drinker status, and number of medications used (checked against packages). Weight, height, and waist circumference were measured at home under standardized conditions, and BMI (weight in kilograms divided by the square of height in meters) was calculated.(40) Information on physical activity, in the household and during leisure time, was collected with the questionnaire used in the EPIC-Spain cohort.(41) Study participants also reported if they had suffered from any of the following physician-diagnosed prevalent conditions: chronic respiratory

disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, and depression requiring treatment.

Statistical analysis

The association between ultra-processed food intake and the risk of frailty was summarized with odds ratios (ORs) and their 95% confidence interval (CI) obtained from logistic regression. The percentage of total energy intake ingested from ultra-processed foods was classified into sex-specific quartiles and the lowest quartile was used as reference. Considering the percentage of total energy intake from ultra-processed foods we take into account the high energy density of these foods. We replicated the same analyses expressing ultra-processed food consumption in gram per day/body weight (g/Kg), to consider the body size as well as the ultra-processed food intake with low energy contribution. The *p* for linear trend was calculated by using quartiles as a continuous variable.

Sociodemographic, lifestyle, and morbidity variables associated with frailty in the literature, were considered as potential confounders. Thus, three logistic models were built with progressive levels of adjustment: the first one was adjusted for sex and age (60–69, 70–79, 80 and older); the second one additionally adjusted for educational level (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (current, former, never smoker), and ex-drinker status; model 3 was also adjusted for chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, depression requiring treatment, and number of medications used. Among robust participants at baseline, we also tested the

association between ultra-processed food intake and each frailty criterion, using the same type of modeling.

We assessed the ultra-processed food intake by food groups, expressed as a percentage of total energy. Food groups were classified into sex-specific quartiles except when: a) the intake of ultra-processed foods from a food group occurred in less than 50% of the subjects, in which variables were classified into sex-specific tertiles; b) when it occurred in less than 25% of the participants, ORs (95% CI) were calculated between participants who consumed the food group compared to those who did not.

Statistical significance was set at two-sided p of <0.05. The analyses were performed with Stata/SE, version 14.1 (StataCorp, College Station, TX).

Results

The mean age of the 1,822 participants was 68.7, and 51.3% were women. On average, the contribution of ultra-processed food intake to total energy intake was 19.3% (from 0% to 79.7%). We also estimated total energy intake across increasing quartiles of ultra-processed food consumption, and a wide variation was found in the relative contribution of each NOVA category to total energy intake among quartiles of ultra-processed food intake (Supplementary Figure 1). Given that energy intake from these foods was lower in men (17.7%) than in women (20.7%), exposure variables were modeled as sex-specific quartiles. Compared with participants in the lowest quartile of ultra-processed food intake, those in the highest quartile consumed more

total energy, were more often ex-drinkers, had depression more frequently, but suffered from osteoarthritis/arthritis less frequently (Table 1).

Over a mean follow-up of 3.5 years, 132 cases of frailty were ascertained. In the fully adjusted analyses, the ORs (95% CI) of frailty risk across quartiles of ultra-processed food intake, expressed as a percentage of total energy, with respect to the first quartile were: 1.52 (0.78-2.96), 2.98 (1.62-5.50), and 3.67 (2.00-6.73); *p* for linear trend: <0.001. The corresponding ORs (95% CI) when ultra-processed foods were expressed in g/Kg were: 2.42 (1.32-4.41), 2.48 (1.37-4.49), and 2.57 (1.41-4.70); *p* for linear trend: 0.004 (Table 2, Model 3).

When each frailty criterion was considered, the greatest OR (95% CI) was the one for unintentional weight loss, which was 3.33 (1.86-5.97) for the highest vs the lowest quartile of ultra-processed food intake, expressed as a percentage of total energy; *p* for linear trend: <0.001. Results had the same direction for other frailty components (low physical activity and slowness), and were also consistent when ultra-processed food intake was measured in g/Kg (Table 3).

Main food groups contributing to ultra-processed food intake were meat and meat products (17.8%), cakes and pastries (12.4%), cookies (11.9%), yogurts and fermented milks (9.5%), jams and confectionary (8.9%), and pre-cooked dishes (7.4%). The highest versus the lowest tertiles of ultra-processed yogurts and fermented milks (natural and non-sugary yogurts are not included in this category), cakes and pastries, as well as non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks) were

303 significantly associated with incident frailty. For cookies, dairy desserts, meat and
304 meat products, sauces and dressings, pre-cooked dishes, and soft drinks the
305 associations were in the same direction, but no statistical significance was achieved
306 (Figure 1).

307 Similar results were obtained in sensitivity analyses that removed unintentional
308 weight loss from the frailty criteria, when age was expressed as a continuous variable,
309 and when physical activity and total energy intake were considered as potential
310 confounders.

311 **Discussion**

312 After 3.5 years of follow-up, ultra-processed food intake was associated with an
313 increased risk of frailty in a cohort of a non-institutionalized population aged 60 or
314 older from Spain. Participants in the highest quartile of total energy intake from ultra-
315 processed foods have a tripled risk of incident frailty than participants in the lowest
316 quartile of consumption.

317 A recent systematic review confirms the importance of both quantitative (e.g. amount
318 of energy intake) and qualitative factors (e.g. nutrient quality) in the development of
319 frailty syndrome in the elderly.(42) Ultra-processed foods are palatable, ready to eat,
320 and with a very high energy density.(14, 18-20) These characteristics make ultra-
321 processed foods more likely to be over-consumed, which may consequently increase
322 total dietary energy intake among consumers. Likewise, ultra-processed food
323 consumption has been associated with a poorer diet quality (less fiber, fruit, and

vegetable intake), that also contributes to frailty.(43, 44) Results are in line with previous analyses of this cohort, in which a high adherence to the Mediterranean diet (low in ultra-processed foods) was inversely related to incident frailty.(30) Also, a Prudent dietary pattern showed an inverse dose-response association with frailty incidence, while a Westernized dietary pattern had a positive association with some of their components (such as high consumption of dairy products as well as red and processed meat).(10) In both cases, the intake of dairy products and red meat was detrimental.

Our findings concur with those obtained in previous research by Laclaustra et al.(29) Association with frailty was strongest for sugars added during food production, while the intake of sugars naturally appearing in foods was not associated with frailty. The amounts of added sugars from pastries and cookies (15% of the total added sugars) and sugar-sweetened beverages (6%) were positively associated with the risk of frailty. Also, the frailty criterion of "unintentional weight loss" increased dose dependently with added sugars.

Interestingly, yogurts and fermented milks is the food group from ultra-processed foods more positively related to frailty and has important content of animal protein as well as simple sugars and saturated fatty acids.(33)

Regarding yogurts and fermented milks, we found mixed results among studies. It has been previously reported with data from this cohort that the consumption of yogurt and whole milk was linked to a greater risk of frailty. Similar results were obtained when these foods were analyzed separately. However, higher consumption of low-fat

346 yogurt and low-fat milk was not linked to a lower incident frailty.(12) These results
347 are not totally comparable with our findings, where yogurts and fermented milks
348 included as ultra-processed were those sugared, sweetened, flavored milks,
349 independent of their amount of fat content. On the other hand, among 106,772
350 individuals from Sweden, the intake of fermented milk products (such as yogurt, sour
351 milk, and cheese), was associated with lower rates of fracture and mortality in women.
352 Risk reductions in men were modest or non-existent.(45) In the aforementioned study,
353 the endpoint was fracture rates (an outcome highly dependent on calcium intake), not
354 frailty, and the participants were younger than in our cohort. Moreover, the difference
355 between ultra-processed and non ultra-processed dairy products was not made. In the
356 future, a comprehensive assessment of the association between dairy products and
357 health among the elderly is needed. So far today, this relationship is controversial. We
358 suggest new studies considering both, the fat content of dairy products and their
359 processing level before making dietary recommendations.

360 The study´s strengths include its prospective design and the estimation of dietary
361 intakes through a validated dietary history. Trained staff collected a wide range of
362 food items. Other advantages were the adjustment for a large number of potential
363 confounders and the robustness of the results that did not change substantially after
364 running several sensitivity analyses. Some limitations should be noted. First, as in
365 most nutritional epidemiology studies, a certain recall bias cannot be ruled out
366 because diet was self-reported. Second, although there was agreement among authors
367 about NOVA classification of our participants' diet, certain ultra-processed food
368 misclassification cannot be ruled out. Third, the number of frailty cases was relatively

small, but we still found quite strong associations. Fourth, the low number of consumers in some ultra-processed food groups (such as breakfast cereals, dairy desserts, and soft drinks) may not allow to achieve statistical significance in those groups. Finally, although we adjusted for the potential confounders, some residual confounding may persist.

Conclusions and Implications

In conclusion, in this prospective study of community-dwelling older adults from Spain, a greater intake of ultra-processed foods, yogurts and fermented milks, cakes and pastries, as well as non-alcoholic beverages (instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks), was associated with an increased risk of frailty. This association was strong and independent of sociodemographic, lifestyle, and morbidity factors. These findings add evidence for the restriction of ultra-processed foods, and the promotion of fresh or minimally processed foods in the older adult population.

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Table 1. Baseline characteristics of the cohort participants according to quartiles of ultra-processed food consumption as a percentage of total energy (% energy), in the Seniors-ENRICA cohort study. (N=1,822)

	Ultra-processed food consumption (% energy)				
	Q1 (lowest) (n=456)	Q2 (n=455)	Q3 (n=456)	Q4 (highest) (n=455)	*p* for linear trend
Total energy (Kcal/d), mean ± SD	1903 ± 525	2003 ± 562	2040 ± 529	22201 ± 606	<0.001
Ultra-processed food consumption (% energy), mean ± SD	6.5 ± 3.1	14.1 ± 2.5	21.7 ± 3.1	34.9 ± 7.7	<0.001
Ultra-processed food consumption (grams per d), mean ± SD	116 ± 88	216 ± 133	294 ± 157	417 ± 192	<0.001
Weight (Kg), mean ± SD	73.3 ± 12.6	74.7 ± 12.6	74.0 ± 13.0	75.2 ± 13.0	0.067
Ultra-processed food consumption (g/Kg), mean ± SD	1.6 ± 1.2	3.0 ± 1.9	4.1 ± 2.2	5.7 ± 2.7	<0.001
Age (y), mean ± SD	68.9 ± 6.2	68.6 ± 6.1	68.6 ± 6.5	68.6 ± 6.6	0.539
Educational level, (%)					0.290
No formal education	21.7%	20.4%	21.1%	22.7%	
Primary	24.1%	24.6%	22.4%	28.6%	
Secondary o higher	54.2%	55.0%	56.6%	48.8%	
Marital status, (%)					0.068
Single	7.7%	7.0%	5.7%	6.8%	
Married	74.1%	73.2%	73.5%	69.7%	
Divorced	2.4%	3.1%	3.1%	4.4%	
Widower	15.8%	16.7%	17.8%	19.1%	
Tobacco consumption, (%)					0.102
Current smoker	10.3%	9.0%	14.9%	12.1%	
Former smoker	31.6%	30.8%	28.5%	32.8%	
Never smoker	58.1%	60.2%	56.6%	55.2%	
Ex-drinker status, (%)	7.2%	9.2%	9.0%	13.0%	0.006
Chronic conditions, (%)					
Chronic respiratory disease	7.5%	8.1%	7.9%	6.8%	0.697
Coronary Disease	1.5%	1.1%	1.3%	1.8%	0.772
Stroke	1.5%	1.1%	0.4%	0.7%	0.109
Osteoarthritis/ arthritis	51.1%	48.4%	44.5%	44.4%	0.022
Cancer	1.8%	1.8%	2.2%	1.8%	0.875
Depression requiring treatment	4.8%	7.7%	7.9%	9.2%	0.015
Number of medications per day, mean ± SD	1.9 ± 1.9	1.9 ± 1.8	2.1 ± 2.1	1.9 ± 1.9	0.597

SD: Standard Deviation. Quartiles ranges are: Men: 0% to 9.55% for Q1; 9.56% to 15.80% for Q2; 15.81% to 24.28% for Q3; and 24.29% to 57.78% for Q4. Women: 0% to 12.01% for Q1; 12.02% to 9.24% for Q2; 19.25% to 27.84% for Q3; and 27.85% to 79.66% for Q4.

Table 2. Frailty risk according to ultra-processed food consumption quartiles in the Seniors-ENRICA cohort study expressed as a percentage of total energy (% energy) or as gram per day/weight of each subject (g/Kg). N=1,822.

	Ultra-processed food consumption (% energy)				
	Q1 (lowest)	**Q2**	**Q3**	**Q4 (highest)**	***p* for linear trend**
N	456	455	456	455	
Frailty	17	24	43	48	
Model 1, OR (95% CI)	1 (Ref.)	1.51 (0.79-2.89)	2.90 (1.60-5.26)	3.22 (1.79-5.79)	<0.001
Model 2, OR (95% CI)	1 (Ref.)	1.53 (0.80-2.95)	2.92 (1.61-5.33)	3.52 (1.94-6.38)	<0.001
Model 3, OR (95% CI)	1 (Ref.)	1.52 (0.78-2.96)	2.98 (1.62-5.50)	3.67 (2.00-6.73)	<0.001
	Ultra-processed food consumption (g/Kg)				
	Q1 (lowest)	**Q2**	**Q3**	**Q4 (highest)**	***p* for linear trend**
N	456	455	456	455	
Frailty	20	36	39	37	
Model 1, OR (95% CI)	1 (Ref.)	2.23 (1.25-4.00)	2.21 (1.24-3.94)	2.17 (1.22-3.89)	0.017
Model 2, OR (95% CI)	1 (Ref.)	2.36 (1.31-4.24)	2.32 (1.30-4.14)	2.38 (1.32-4.28)	0.008
Model 3, OR (95% CI)	1 (Ref.)	2.42 (1.32-4.41)	2.48 (1.37-4.49)	2.57 (1.41-4.70)	0.004

Notes: CI = confidence interval; OR = odds ratio. The p values for the ORs were obtained using logistic regression.
Model 1: Logistic regression model adjusted for sex, age (60–69, 70–79, ≥80 years).
Model 2: As in Model 1 and additionally adjusted for level of education (primary or less, secondary, university), marital status (single, married, divorced, widower), tobacco consumption (current, former, never smoker), and ex-drinker status (yes, no).
Model 3: As in Model 2 and additionally adjusted for chronic respiratory disease (yes, no), coronary disease (yes, no), stroke (yes, no), osteoarthritis/arthritis (yes, no), cancer (yes, no), depression requiring treatment (yes, no), and number of medications used (continuous).

Table 3. Odds ratios of frailty components according to ultra-processed food consumption quartiles in the Seniors-ENRICA cohort study expressed as a percentage of total energy (% energy) or as gram per day/weight of each subject (g/Kg).

	Ultra-processed food consumption (% energy)				
	Q1 (lowest)	**Q2**	**Q3**	**Q4 (highest)**	***p* for linear trend**
Low physical activity, OR (95% CI)	1 (Ref.)	1.09 (0.75-1.60)	1.35 (0.94-1.96)	1.35 (0.93-1.96)	0.066
Slowness, OR (95% CI)	1 (Ref.)	0.82 (0.53-1.24)	1.64 (1.13-2.39)	1.74 (1.19-2.54)	<0.001
Unintentional weight loss, OR (95% CI)	1 (Ref.)	1.77 (0.94-3.32)	3.15 (1.75-5.66)	3.33 (1.86-5.97)	<0.001
Muscle weakness, OR (95% CI)	1 (Ref.)	1.05 (0.78-1.43)	1.11 (0.82-1.51)	1.06 (0.78-1.44)	0.661
Self-reported exhaustion, OR (95% CI)	1 (Ref.)	1.12 (0.74-1.70)	1.15 (0.76-1.75)	1.21 (0.80-1.83)	0.379
	Ultra-processed food consumption (g/Kg)				
	Q1 (lowest)	**Q2**	**Q3**	**Q4 (highest)**	***p* for linear trend**
Low physical activity, OR (95% CI)	1 (Ref.)	1.36 (0.93-2.00)	1.41 (0.96-2.07)	1.87 (1.28-2.74)	0.002
Slowness, OR (95% CI)	1 (Ref.)	1.07 (0.71-1.63)	1.79 (1.21-2.63)	2.02 (1.37-2.97)	<0.001
Unintentional weight loss, OR (95% CI)	1 (Ref.)	1.66 (0.93-2.97)	2.28 (1.31-3.94)	2.46 (1.42-4.26)	<0.001
Muscle weakness, OR (95% CI)	1 (Ref.)	0.82 (0.61-1.12)	1.05 (0.78-1.42)	1.06 (0.78-1.44)	0.426
Self-reported exhaustion, OR (95% CI)	1 (Ref.)	1.26 (0.85-1.88)	0.93 (0.61-1.40)	1.03 (0.68-1.56)	0.734

OR: Odds ratio. CI: Confidence interval. Analyses adjusted as in model 3.

LEGENDS FOR FIGURES

Figure 1. Odds Ratio of frailty and 95% Confidence Intervals (95% CI) for quartile 4 (highest intake) of groups of ultra-processed food consumption as a percentage of total energy (% energy) or as caloric density (g/1000 kcal or mg/1000 kcal) versus quartile 1 (lowest intake), in the Seniors-ENRICA cohort study. N=1,822.

When the intake of ultra-processed food from a specific food group occurred in less than 50% of the participants, the Odds Ratio (95% CI) was calculated among tertiles (as in breads, cookies, cakes and pastries, yogurts and fermented milks, jams and confectionary, and non-alcoholic beverages). When the intake of ultra-processed food from a specific food group occurred in less than 25% of the participants, the Odds Ratio (95% CI) was calculated between subjects who consumed the food compared to those who didn't (as in breakfast cereals, dairy desserts, and soft drinks). *P* for linear trend was calculating using quartiles as a continuous variable. Model was adjusted for sex, age, level of education, marital status, tobacco consumption, ex-drinker status, chronic respiratory disease, coronary disease, stroke, osteoarthritis/arthritis, cancer, depression, and number of medications used.

*Non-alcoholic beverages group includes instant coffee and cocoa, packaged juices, and other non-alcoholic drinks, excluding soft drinks (see Supplementary Table 1).

Consumption of ultra-processed foods and mortality: a national prospective cohort in Spain

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Abbreviations and Acronyms

ENRICA= Study on Nutrition and Cardiovascular Risk in Spain (*Estudio de Nutrición y Riesgo Cardiovascular en España*)

ABSTRACT

Objective: To assess the prospective association between ultra-processed food consumption and all-cause mortality and to examine the effect of theoretical iso-caloric non-processed foods substitution.

Patients and Methods: A population-based cohort of 11,898 individuals (mean age 46.9 years, and 50.5% women) were selected from the ENRICA study, a representative sample of the non-institutionalised Spanish population. Dietary information was collected by a validated computer-based dietary history and categorized according to their degree of processing using NOVA classification. Total mortality was obtained from the National Death Index. Follow-up lasted from baseline (2008-2010) to mortality date or December 31th, 2016, whichever was first. The association between quartiles of consumption of ultra-processed food and mortality was analyzed by Cox models adjusted for the main confounders. Restricted cubic-splines were used to assess dose-response relationships when using iso-caloric substitutions.

Results: Average consumption of ultra-processed food was 385 grams/d (24.4% of the total energy intake). After a mean follow-up of 7.7 years (93,599 person-years), 440 deaths occurred. The hazard ratio (95% confidence interval) for mortality in the highest versus the lowest quartile of ultra-processed food consumption was 1.44 (1.01 to 2.07; *P*-trend=.03) in % of energy and 1.46 (1.04 to 2.05, *P*-trend=.03) in grams d/kg. Iso-caloric substitution of ultra-processed food with unprocessed or minimally processed foods was associated with a significant non-linear decrease in mortality.

Conclusion: A higher consumption of ultra-processed food was associated with higher mortality in the general population. Furthermore, the theoretical iso-caloric substitution ultra-processed food by unprocessed or minimally processed foods would suppose a

reduction of the mortality risk. If confirmed, these findings support the necessity of the development of new nutritional policies and guides at national and international level.

Study registration: ClinicalTrials.gov ID: NCT01133093

INTRODUCTION

Processing of food arose as a need to improve food availability, safety, digestibility, transportability, and storage life [1, 2]. In the last decades, processing of food and food supplies have increased broadly providing ready-to consume processed products that can be distributed all around the world [3]. Several food classifications have been proposed to quantify the nature and the extent of processed food intake when measuring it in populations [4]. The most extreme category corresponds to the ultra-processed foods, that are formulations made mostly or entirely from substances derived from foods and additives, with little if any whole food [4].

Compared to the rest of the diet, ultra-processed foods often have a higher content of total fat, saturated fat, added sugars, and salt, along with a low amount of fiber, and low vitamin density [5-7]. Besides that, they may contain neo-formed contaminants derived from physical, chemical, and biological processes, along with substances from packaging and additives [8, 9]. Moreover, epidemiological evidence has demonstrated that ultra-processed food consumption is associated with poorer diet quality [10, 11]. On the other hand, ultra-processed foods are very palatable and habit-forming, convenient, microbiologically safe, affordable, as well as aggressively advertised and marketed [12, 13]. National household food purchase surveys and national dietary surveys have informed on ultra-processed food intake in some Western countries including USA [14], Canada [15], New Zealand [5], several European countries, [16, 17] and some South American countries, such as Brazil, [18] and Chile [19]. It has been estimated that ultra-processed food intake is increasing and it currently contributes between 25 to 50% of total energy intake [20].

These shifts in dietary habits have been parallel to an increase in chronic diseases, beyond what was expected for a subject's chronological age [21] and ultra-processed food consumption may play a role in this phenomenon [22]. Indeed, several longitudinal studies

observed that ultra-processed food intake was associated with a higher incidence of dyslipidemia [23], hypertension [24], and cancer [25]. Moreover, both cross-sectional [26, 27] and longitudinal studies [28] found a relationship with obesity. However, to date, a comprehensive assessment of the association between ultra-processed food consumption and mortality has not been reported.

This study, conducted on a representative sample of the non-institutionalized adult population of Spain, aimed to elucidate, for the first time, the association of ultra-processed food consumption as a whole with all-cause mortality. Moreover, we have examined the effect of theoretical iso-caloric non-processed foods substitution on this relationship.

MATERIAL AND METHODS

Study Population

Data were taken from the Study on Nutrition and Cardiovascular Risk in Spain (ENRICA), whose methods have been reported elsewhere [29]. In brief, 12,948 individuals were selected between June 2008 and October 2010 by stratified cluster sampling, to ensure that they were a representative sample of the non-institutionalized population of Spain aged ≥18. First, the sample was stratified by province and size of the municipality. Second, clusters were selected randomly in two stages: municipalities and census sections. Finally, the households within each section were selected by random telephone dialing. Participants in the households were selected proportionally to the sex and age distribution of the Spanish population.

Trained and certified personnel collected information in three sequential stages: a) a phone interview to obtain data on sociodemographic factors, health behaviors, self-rated health, and morbidity; b) a first home visit to collect blood and urine samples, and c) a second home visit to perform a physical examination, and to obtain habitual diet by

using a computerized dietary history. More information about the sample collection process has been already published [29].

Written informed consent was obtained from all participants. The study was approved by the Clinical Research Ethics Committees of the La Paz University Hospital in Madrid, and the Hospital Clinic in Barcelona (Spain).

Dietary assessment and extent and purpose of processing of foods

A validated computer-based dietary history (DH-ENRICA) was used to ascertain the participant's habitual consumption of different foods. The DH-ENRICA is a computerized questionnaire administered by a trained interviewer which include 880 foods that can be cooked in 29 different ways and 184 recipes for dishes commonly eaten in Spain or typical of each region. Taking into account weekly frequency of consumption of each food as well as seasonal intake, this dietary history provides an estimate in daily grams of foods that represent the average intake during the preceding year. More details about DH-ENRICA and dietary information collection have been published in Guallar-Castillon et al. [30]. Standard food composition tables from Spain and other countries allowed calculation of the amount of energy and nutrient intake [30].

All recorded food items were classified according to the NOVA food classification based on the extent and purpose of industrial food processing [6]. The rationale underlying the NOVA food classification, the detailed definition of each NOVA food group, and examples of food items classified in each group have been shown elsewhere [4, 18, 31]. Foods were classified into four groups: 1) "unprocessed or minimally processed foods", defined as foods that are of plant or of animal origin consumed shortly after harvesting, gathering, slaughtering or husbanding; or foods that are altered in ways that do not add or introduce any substances, but that may involve subtracting parts of the

food; 2) “processed culinary ingredients”, defined as food products extracted and refined from elements of foods, such as plant oils, animal fats, starches, and sugar; or obtained from nature, (such as salt), that are normally not consumed by themselves; 3) “processed foods”, that are made by adding salt or sugar (or other substances of culinary use such as oil or vinegar) to unprocessed or minimally processed foods, in order to preserve them or to enhance their palatability; and 4) “ultra-processed foods”, which are those that were formulated mostly or entirely from substances derived from foods, with little or even no whole food content. These ingredients include modified starches, hydrogenated oils, protein isolates, and additives whose purpose is to imitate sensorial qualities of unprocessed or minimally processed foods and their culinary preparations, or to disguise undesirable qualities of the final product, such as colorants, flavourings, non-sugar sweeteners, emulsifiers, humectants, sequestrants, and firming, bulking, de-foaming, anti-caking, and glazing agents. The full list of the recorded foods and their NOVA classification is shown in the Supplemental Appendix. It should be noted that a food group can have foods that can be classified in different NOVA categories. For example, in the food group “yogurts and fermented milks”, natural skimmed fermented milk, natural fermented whole milk, natural skimmed yogurt, natural whole yogurt, and kefir were considered in group 1 as “unprocessed or minimally processed foods”; whereas skimmed fermented milk with fruits, whole fermented milk with fruits, skimmed flavoured yogurt, skimmed yogurt with fruits, natural skimmed yogurt with sweetener, whole flavoured yogurt, whole yogurt with fruits, natural whole yogurt with sweetener, whole drinking/liquid yogurt, whole drinking/liquid yogurt with fruit, natural drinking/liquid yogurt with sweetener, and natural Greek yogurt were considered in group 4 as “ultra-processed foods”.

Mortality ascertainment

All-cause mortality from baseline in 2008-2010 to the end of follow-up on December 31st, 2016 was obtained for 99,9% of the cohort. A computerized search was performed in the Spanish National Death Index, which contains information on the vital status of all residents in Spain. Censoring was set at the date of death or at the end of follow-up whichever occurred first.

Covariates

Sex, age, educational level (no formal education, primary, and secondary or higher) were recorded. Individuals also reported if they lived alone or not; if they were current, former or never smokers; and if they were former drinkers. Physical activity was recorded using the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort questionnaire. A Physical Activity Index was established based on a cross-tabulation of occupational, household, and recreational activities, categorizing individuals into four levels of activity: inactive, moderately inactive, moderately active, and active [32] Time spent watching TV and time devoted to other sedentary activities (computer use, reading, commuting, and listening to music) were also registered, in hours per week [33]. Finally, the amount of medication per day, as well as the presence of chronic conditions diagnosed by a physician (chronic respiratory disease, coronary heart disease, stroke, heart failure, osteoarthritis, cancer, and depression requiring treatment) were also self-reported.

Statistical analysis

Of the 12,948 study participants, a total of 1050 was excluded because of: inconsistent dietary data (total daily energy intake outside the range of 800–5000 kcal in men, or 500–4000 in women) (n=60), missing information on diet (n=887), or covariates

(n=103). Thus, the analyses were conducted with 11,898 individuals (5,890 men and 6,008 women).

For each participant, the proportion of total energy from ultra-processed foods was calculated, and the total sample was divided into quartiles (cut-off points were set at 14.1%, 23.0%, and 33.1%). Cox proportional hazards models were used to assess the association between quartiles of ultra-processed food intake (in % of total energy intake) and mortality. The first quartile (lowest intake) was used as the reference. Analyses were weighted to account or the complex sampling design, and the variances were also corrected to calculate appropriate 95% confidence intervals (CI). Linear trend was calculated using quartiles of ultra-processed food as a continuous variable. Schoenfeld residuals were plotted against time to detect violations of the proportional hazard assumption. Finally, ultra-proceed foods were also expressed in grams per day/weight of the participant. Body weight was assessed by using electronic scales (model Seca 841: Seca Deutschland, Hamburg, Germany, precision to 0.1 kg)

Cox models were built with 4 successive levels of additional adjustments: Model 1 was adjusted for age and sex; Model 2, for socioeconomic factors (educational level and living alone); Model 3, for lifestyle factors (smoking status, former drinker, physical activity, time watching TV, and time devoted to other sedentary activities); and Model 4 was additionally adjusted for clinical factors (number of medications per day and presence of chronic conditions). We also performed several sensitivity analyses.

We built iso-caloric substitution models that simultaneously included energy intake, the percentages of energy derived from ultra-processed foods, from processed culinary ingredients, and processed food or unprocessed or minimally processed foods, as appropriate, as well as other potential confounding variables. Non-linear relations were identified using restricted cubic spline analyses with 3 knots or inflection points placed

at the 10^{th}, 50^{th}, and 90^{th} percentiles. The coefficients from these models can be interpreted as the estimated association of substituting a certain percentage of energy from ultra-processed foods with the equivalent energy from processed foods or unprocessed or minimally processed foods while holding constant the intake of total energy as well as the energy from the corresponding non-replaced NOVA groups [34].

Secondary analyses were performed for nutrients intake from ultra-processed foods (as a percentage of total energy or as caloric density), considering the main food groups contributing to ultra-processed food intake. These variables were divided into quartiles, tertiles or median according to the number of participants with intake in the corresponding categories and, always using the lowest category as a reference. Analyses were performed with Stata software version 14 for Windows (StataCorp LP), and statistical significance was set at $P<.05$. The survey command was used in the analyses to take into account the complex sampling.

RESULTS

The cohort comprised of 11,898 subjects (mean age 46.9 and 50.5% women) who had an average consumption of ultra-processed food of 385 grams/d, corresponding to 24.4% of the total energy intake. Those in the first quartile of ultra-processed foods consumed a mean of 8.7% (mean=156 gr), versus 42.8% (mean=641 gr) consumed by those in the highest quartile (Supplemental Figure 1). After a mean of 7.7 years and 93,599 person-years followed up, 440 deaths occurred.

Subjects that consumed more ultra-processed foods also ingested more energy, were younger, more frequently current smokers, more educated, had a higher activity index, watched fewer hours of TV, but dedicated more time to other sedentary activities; took less medication per day, and had lower prevalence of stroke, heart failure, osteoarthritis,

and cancer, but more depression (Table 1). They also had a higher intake of carbohydrates, simple sugars, total fat, saturated fatty acids, polyunsaturated fatty acids, trans fatty acids, and sodium (Supplemental Table 1). The main food groups contributing to ultra-processed food intake were meat and meat products (17.1%), cakes and pastries (13.6%), cookies (9.2%), yogurts and fermented milk (8.8%), jams and confectionery (7.4%), and pre-cooked dishes (7.1%). These food groups had a different relative contribution among ultra-processed quartiles (Supplemental Table 2).

In all models, participants in the highest quartile of ultra-processed food intake had higher mortality risk when compared with those in the lowest quartile; in model 4, the hazard ratio (95% CI) for mortality was 1.44 (1.01 to 2.07), *P* for trend=.03. The corresponding mortality risk when ultra-processed food consumption was expressed in grams per day/weight was 1.46 (1.04 to 2.05), *P* for trend=.03 (Table 2). Sensitivity analyses (Supplemental Table 3) provided similar results.

In model 4, the hazard ratio of the iso-caloric substitution of ultra-processed foods with processed foods was below 1 but did not achieve statistical significance (Figure 1A). However, when replaced with unprocessed or minimally processed foods an inverse and non-linear dose-response was observed (*P* for non-linearity=.04), starting to be significant when replacing 8.2% of ultra-processed food consumption (Figure 1B).

When comparing the highest vs. the lowest quartile of nutrients intake from ultra-processed foods, consumption of total carbohydrates, simple sugars, saturated fatty acids, and trans fatty acids contributed the most to increased mortality risk, although only trans fatty acids reached statistical significance [HR 1.39, 95% CI 1.00 to 1.92; *P* for trend=.05] (Supplemental Figure 2). When analyzing ultra-processed food consumption by food groups, the intake of yogurts and fermented milks (those classified as ultra-processed), cakes and pastries, and cookies contributed the most to an increased

mortality risk, but it only reached statistical significance for yogurts and fermented milks [HR: 1.37, 95% CI 1.02-1.86] with a non-significant trend (*P* for linear trend=.09). The other groups of ultra-processed foods (breads, breakfast cereals, dairy desserts, meat and meat products, jams and confectionery, sauces and dressings, soft drinks, packaged fruit juices, and nectars) were not associated with increased risk of mortality. (Supplemental Figure 3).

DISCUSSION

Principal findings

In this prospective study, based on a representative sample of the non-institutionalized adult population of Spain, an increased intake of ultra-processed food was associated with higher mortality after 7.7 years of follow-up. Participants in which ultra-processed food intake contributed more than 33% of total energy intake had a 44% higher all-cause mortality risk compared to those in which ultra-processed food intake contributed up to 14%.

Interpretation and comparison with other studies

To the best of our knowledge, this is the first prospective epidemiological study performed in a large and representative national cohort that evaluated the association between ultra-processed food consumption (as a whole) and risk for all-cause mortality. In Spain, consumption of ultra-processed food intake was 24.4% of total dietary calories, being consistent with previous data from the DAFNE databank showing that 20.3% of total purchased dietary energy came from ultra-processed foods [27]. Spain is a country with low ultra-processed food consumption when compared with other Western countries, such as Canada (61.7%) [15], the USA (57.9%) [14], the UK (53%) [35], and France (35.9%) [16] or developing countries such as Brazil (29.6%) [18]. This could be explained as cooking at home is part of the Mediterranean diet (that is also rich in unprocessed or

minimally processed foods) [36-38]. However, it is also known that the Spanish population has been drifting away from this traditional pattern to adopt a less healthy diet [39], especially among young people [40], supporting the estimates that ultra-processed foods consumption will continue to increase [20].

The association of some groups of ultra-processed foods with mortality has been studied previously. In the US, positive associations were found between fast food and sugar-sweetened beverage consumption (SSBs) and mortality [41]. Also, a recent meta-analysis showed a non-linear 7% higher risk with an increased intake of SSBs up to 250 mL/d [42]. An increased risk of mortality has also been shown with meats and processed meats [42-44] Regarding dairy products; studies showed controversial results. Although a recent meta-analysis of 29 prospective cohort studies demonstrated neutral associations between milk and dairy products and mortality, there was not a distinction between processed and ultra-processed foods [45]. In the present analysis, sugared-, sweetened-, flavored-, and additive added- dairy products were considered as ultra-processed. On the contrary, milk, plain yogurts, and fresh and cured cheeses were not included in this category. Our results suggest that the intake of ultra-processed yogurts and fermented milks is associated with increased mortality, reinforcing the idea of considering the extent of processing when studying dairy product. Moreover, the obtained iso-caloric replacement results are supported by different studies that used national household data to estimate the contribution of dietary trends for risk of cardiovascular disease and all-cause mortality. In the UK, a reduction of 13% of cardiovascular disease mortality was projected by 2030 if dietary intake of ultra-processed and processed foods were entirely replaced with unprocessed or minimally processed foods [46]. In Brazil, using a similar approach, an 11% cardiovascular mortality reduction was estimated if ultra-processed

food were reduced by 50%, and substituted with unprocessed or minimally processed foods, plus an additional 50% reduction in processed culinary ingredients [47].

How ultra-processed food consumption increases the risk of all-cause mortality may depend on a number of factors. Ultra-processed foods have a high energy density that is less satiating, and highly accessible [6, 31, 48], being prone to causing inadvertent overconsumption, which has also been associated with mortality [49]. Moreover, ultra-processed foods are industrial formulations containing high quantities of saturated fatty acid, trans-fatty acids, hydrogenated oils, starches, free sugars, and salt, plus food additives used to imitate the sensory qualities of natural foods, or to disguise undesirable qualities of the final product, such as colorants, flavourings, artificial sweeteners and emulsifiers [5, 9]. Harmful effects of some of these nutritional compounds have been widely studied. Trans-fatty acids intake has been related to an increase in all-cause mortality risk in several populations [50, 51] as well as total sugar intake [52]. The association of saturated fatty acids intake and mortality is controversial [53, 54], highlighting the need to take into account the food sources of saturated fatty acids [55]. Also, a meta-analysis of 23 cohort studies found a U-shaped association between sodium intake and mortality [56]. In our cohort, the separate analysis of these nutrients from ultra-processed foods increase the risk of all-cause mortality, although only trans-fatty acids reached a significant association. These results support the application of food processing classification beyond conventional food classifications, as well as the report on single nutrients [5]; since the concept of ultra-processed foods does not just focus attention on these "traditional" harmful nutrients but to a wider range of food additives contained in this type of foods. Although food additives safety is rigorously controlled by the European authorities [57], the detrimental health effects in the long term and the potential interaction consequences between the different compounds are largely

unknown [9]. Finally, consumption of ultra-processed foods was also associated with a poorer diet quality (with less consumption of fiber, fruit, and vegetables) [10, 11] and unhealthy lifestyle (smoking and sedentary behaviors) [16, 58], all contributing to increased mortality [12, 42, 59]. However, further mechanistic studies to know how and to which extent ultra-processed foods may impact on health should be performed.

Strengths and limitations of the study

This study has some strengths including its prospective design, and a relatively large sample size representative of the adult Spanish population, which broaden the generalization of the results. Food consumption was collected in detail with a validated dietary history including a wide variety of foods and their cooking methods [30]. Finally, many confounding factors were considered in the analysis. However, there are some limitations as well. First, dietary information was obtained only at baseline assuming no time changes in dietary intake, and probably underestimating the real impact of ultra-processed foods on mortality. Second, although there was a consensus between authors about NOVA classification of the foods obtained by the dietary history, some degree of misclassification among ultra-processed food categories cannot be ruled out. In this regard, we were aware that NOVA classification has been criticized (mainly because of the broad definition of ultra-processed-food) [60], but nowadays is the most used and recommended to classified ultra-processed foods in public health nutrition [20]. Third, the relatively small number of deaths precluded meaningful analyses by specific causes of death. Last, a certain degree of residual confounding can still exist, and non-differential misclassification affecting both, exposure and disease, can still operate, leading to a dilution bias of the true effect.

Conclusions and policy implications

In conclusion, an increased intake of ultra-processed food was associated with a higher risk of mortality. Moreover, the theoretical iso-caloric substitution ultra-processed food by unprocessed or minimally processed foods would suppose a reduction of the mortality risk. These results are needed to be confirmed by other large-scale, population-based prospective studies in different countries and settings. Further studies are also needed to identify the specific foods that mostly account for this association as well as to investigate possible relationships with specific causes of death. However, our findings support the epidemiological evidence about the harmful effects of ultra-processed foods, and thus, the necessity to implement actions, such as the development of new nutritional policies and guides, both for the population and the industry. Ultra-processed foods intake can be considered one of the major challenges that governments will have to face in the next decades, and one of the main opportunities for nutritional prevention.

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Table 1. Baseline characteristics of the cohort participants according to quartiles of ultra-processed food consumption (% of energy), in the ENRICA Study (2008-10).

		Quartiles of ultra-processed food consumption (% total energy)				
	All subjects (N=11,898)	**Q1 (lowest) (n=2,976)**	**Q2 (n=2,974)**	**Q3 (n=2,974)**	**Q4 (highest) (n=2,974)**	***P* for linear trend**
Total energy (Kcal/d), mean ± SE	2,173.9 ± 9.0	1,970.0 ± 13.3	2,078.0 ± 13.4	2,263.5 ± 17.4	2,378.9 ± 15.8	<.001
Ultra-processed food consumption (% of energy), mean ± SE	24.47 ± 0.17	8.68 ± 0.08	18.60 ± 0.05	27.82 ± 0.06	42.83 ± 0.19	<.001
Ultra-processed food consumption (grams per d), mean ± SE	384.70 ± 4.30	155.50 ± 2.90	304.70 ± 5.10	436.90 ± 6.20	641.20 ± 9.00	<.001
Weight (Kg), mean ± SE	73.80 ± 0.20	74.60 ± 0.30	74.10 ± 0.40	73.60 ± 0.30	73.00 ± 0.40	<.001
Ultra-processed food consumption (grams per d/weight), mean ± SE	5.34 ± 0.06	2.12 ± 0.04	4.18 ± 0.07	6.08 ± 0.09	9.01 ± 0.13	<.001
Women, (%)	50.5%	49.1%	51.8%	50.1%	51.0%	.39
Age (y), mean ± SE	46.9 ± 0.27	54.93 ± 0.40	49.40 ± 0.38	44.60 ± 0.44	38.79 ± 0.40	<.001
Educational level, (%)						<.001
No formal education	30.0%	40.5%	31.2%	26.1%	22.1%	
Primary	42.0%	32.6%	40.5%	45.1%	49.7%	
Secondary or higher	28.1%	26.9%	28.4%	28.8%	28.2%	
Living alone, (%)	7.8%	9.0%	8.8%	7.1%	6.2%	<.001
Smoking status, (%)						<.001
Current smoker	27.5%	24.7%	27.0%	28.4%	30.0%	
Former smoker	24.7%	30.8%	25.7%	22.9%	19.6%	
Never smoker	47.8%	44.6%	47.3%	48.7%	50.4%	
Former drinker, (%)	5.5%	5.3%	5.5%	5.8%	5.4%	.93
Physical Activity Index, (%)						<.001
Inactive	27.7%	33.2%	28.6%	25.6%	23.4%	
Moderately inactive	33.8%	35.2%	33.3%	33.3%	33.3%	
Moderately active	23.0%	20.1%	23.7%	24.2%	23.9%	
Active	15.6%	11.6%	14.4%	17.0%	19.4%	
Time watching TV (h/week), mean ± SE	13.67 ± 0.12	14.43 ± 0.24	13.85 ± 0.23	13.35 ± 0.21	13.16 ± 0.23	<.001
Time devoted to other sedentary activities (h/week), mean ± SE	15.52 ± 0.16	13.69 ± 0.29	15.11 ± 0.26	16.24 ± 0.33	17.03 ± 0.28	<.001
Number of medications per day, mean ± SE	0.84 ± 0.02	1.18 ± 0.04	0.95 ± 0.04	0.71 ± 0.03	0.53 ± 0.03	<.001
Chronic conditions, (%)						
Chronic respiratory disease	6.4%	6.3%	6.8%	6.5%	5.9%	.72
Coronary heart disease	0.6%	0.9%	0.6%	0.5%	0.4%	.15
Stroke	0.4%	0.7%	0.6%	0.2%	0.3%	.03
Heart Failure	1.5%	1.7%	1.9%	1.1%	1.1%	.03
Osteoarthritis	20.7%	28.2%	24.6%	17.2%	12.9%	<.001
Cancer	1.0%	1.6%	0.9%	0.9%	0.7%	.01
Depression	6.6%	6.4%	7.3%	5.4%	7.5%	.02

SE: Standard error.

Inter-quartiles ranges are: 0% to 14.08% for Q1; 14.09% to 23.00% for Q2; 23.01% to 33.14% for Q3; and 33.15% to 100% for Q4.

Table 2. Mortality risk according to ultra-processed food consumption quartiles in the ENRICA Study. (N= 11,898).

	Quartiles of ultra-processed food consumption (% energy)				
	Q1 (lowest)	**Q2**	**Q3**	**Q4 (highest)**	***P* for linear trend**
n	2,976	2,974	2,974	2,974	
Deaths	158	105	103	74	
Person-years	23308	23378	23438	23475	
Model 1, HR (95% CI)	1 (Ref.)	0.97 (0.73-1.30)	1.28 (0.95-1.73)	1.44 (1.00-2.06)	.02
Model 2, HR (95% CI)	1 (Ref.)	0.98 (0.73-1.31)	1.30 (0.96-1.74)	1.51 (1.05- 2.16)	.01
Model 3, HR (95% CI)	1 (Ref.)	0.94 (0.70-1.26)	1.25 (0.93-1.68)	1.42 (0.99-2.04)	.03
Model 4, HR (95% CI)	1 (Ref.)	0.91 (0.67- 1.23)	1.23 (0.91-1.67)	1.44 (1.01-2.07)	.03
	Quartiles of ultra-processed food consumption (grams per d/weight)				
	Q1 (lowest)	**Q2**	**Q3**	**Q4 (highest)**	***P* for linear trend**
n	2,951	2,950	2,950	2,950	
Deaths	148	125	98	59	
Person-years	23181	23183	23222	23281	
Model 1, HR (95% CI)	1 (Ref.)	1.07 (0.81-1.41)	1.21 (0.91-1.62)	1.48 (1.05-2.10)	.02
Model 2, HR (95% CI)	1 (Ref.)	1.08 (0.82-1.43)	1.23 (0.93-1.65)	1.39 (0.97-1.99)	.01
Model 3, HR (95% CI)	1 (Ref.)	1.08 (0.82-1.43)	1.19 (0.89-1.60)	1.48 (1.06-2.07)	.02
Model 4, HR (95% CI)	1 (Ref.)	1.08 (0.81-1.44)	1.21 (0.89-1.64)	1.46 (1.04-2.05)	.03

Hazard Ratio (HR) and 95% Confidence Intervals (95% CI). Model 1: adjusted for sex and age. Model 2: adjusted as in model 1 plus, educational level, and living alone. Model 3: adjusted as in model 2 plus, smoking status, former drinker, physical activity index, time watching TV, and time devoted to other sedentary activities. Model 4: adjusted as in model 3 plus the number of medications per day, and specific chronic conditions diagnosed by a physician (chronic respiratory disease, coronary heart disease, stroke, heart failure, osteoarthritis, cancer, and depression).

Figure 1. A) Risk of death associated with iso-caloric replacement of ultra-processed food (in % of energy) with processed foods (in % of energy). The y-axis shows the predicted HRs for total mortality, and the x-axis shows the percentage of energy from non-processed food intake as a continuous variable. Lines are restricted cubic splines, showing the shape of the iso-caloric substitution. The black line represents HR, and the dash lines indicate the upper and lower 95% confidence intervals. The knots were located at the 10^{th}, 50^{th} and 90^{th} percentiles (2.28% 6.03% and 11.07% of energy intake, respectively). Adjusted as in model 4. (*P*-nonlinearity= .53). **B) Risk of death associated with iso-caloric replacement of ultra-processed food (in % of energy) with unprocessed or minimally processed foods (in %of energy).** The y-axis shows the predicted HRs for total mortality, and the x-axis shows the percentage of energy from non-processed food intake as a continuous variable. Lines are restricted cubic splines, showing the shape of the iso-caloric substitution. The black line plots the HR, and the dash lines indicate the upper and lower 95% confidence intervals. The knots were located at the 10^{th}, 50^{th} and 90^{th} percentiles (4.52%, 6.89% and 10.3% of energy intake, respectively). Adjusted as in model 4. (*P*-nonlinearity= .04).